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Nutrition and Psyche
*with Special Reference to Experimental Psychodietetics**

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THE INFLUENCE of diet on man's performance capacity and his "mind"—his intellect and personality, his creative capacities, and religious sensitivity—these questions have fascinated men from time immemorial. However, it has been only within very recent times that scientific methods have been extended to the study of nutrition and its effects on psyche.

A variety of quantitative and semiquantitative techniques, available for a description of different aspects of behavior, have been applied in experimental studies on human and animal nutrition. A critical examination of these instruments indicates that they are not fully satisfactory. It may be suggested (and the thesis is not completely facetious) that the precision of our methods is inversely proportional to the real importance of the functions measured, and perhaps also to their sensitivity under nutritional stress. We have seen that self-initiated mental activity has decreased dramatically under conditions of prolonged caloric deprivation, while the measured level of intelligence showed little or no change (Ref. 44, p. 863). And there are numerous other methodological limitations.[15] Yet, awareness of these limitations should not obscure the achievements that have been made in this young segment of the science of nutrition and the need for replacing with facts nutritional folklore and notions of the faddist concerning the psychological effects of foods.

In considering nutrition and behavior, we deal with complex variables. Behavior, as the operational equivalent of "psyche," includes not only the motor functions (such as coordination or speed of movements) but also sensory functions (particularly taste and smell), intelligence, and personality in the narrow sense (with bodily sensations and feeling of well-being, emotions, and motivation).

These two complex systems, nutrition and behavior, may be studied by regarding either the one or the other as the "independent" variable. Thus, on one hand, we have the impact of nutrition (overnutrition, malnutrition, undernutrition) on behavior—and this problem, examined in the experimental setting of the laboratory, has been our concern at Minnesota since 1941. On the other hand, one can consider the psychological factors, individual and social, involved in food selection and food acceptance, in the likes and dislikes, taste preferences and food aversions.

At times, as in dealing with obesity or anorexia nervosa, the simplicity of the "inde-

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pendent-dependent" variable goes by the board. While psychological (emotional) factors may play an "independent" role at the outset and initiate faulty food intake, the developing nutritional defect will, in its turn, affect personality. A seclusion from society, associated with severe grief or lack of belonging, may lead to the adoption of bizarre and unhealthful dietary habits which, in their turn, may result in, say, pellagra, with the development of severe mental disturbances.

Food, Man, and Society

A comprehensive study of the relations between food, nutrition, and behavior requires the bringing together of the disciplines concerned with the composition of food and man's attitudes toward food, with human culture, and with human biology.

Richards,[66] an anthropologist, undertook a comprehensive field study of nutrition in a primitive society focused on the social and cultural functions of food and eating. Her thesis is that nutrition, as a biological process, is more fundamental than sex: "In the life of the individual organism, it (i.e., nutrition) is the more primary and recurrent physical want, while in the wide sphere of human society it determines, more largely than any other physiological function, the nature of social groupings and the form their activities take" (Ref. 66, p. 1).

While "hunger," representing the organism's need for food, is one of the basic biological drives, in human societies food, feeding, and eating acquire values which have little to do with physical survival. B. Malinowski, in his foreword to Richard's volume, stresses the importance of inquiry into the symbolism of food and the culturally determined attitudes toward food. He cites instructive as well as amusing instances of the differences in the "ideology of food," both from his field work in Melanesia and from Europe. The natives of the Trobriand Islands, we are informed, are quite restrained in their food preferences. They abhor their neighbors as cannibals, dog-eaters, and snake-eaters. Their neighbors, in their turn, despise the Trobrianders for showing a poor taste and a lack of discrimination in neglecting such excellent viands as snake, dog, and man.

The symbolism of food is of interest not only to the cultural anthropologist but also, on occasion, to the physician dealing with patients with *anorexia nervosa* (with fear of oral impregnation as one of the underlying motives) and the milder instances of food rejection, or with the opposite problem of overeating.

The psychological factors involved in eating have been systematically examined and described by Selling and Ferraro.[70] There is a brief chapter on the effect of improper diet on behavior and personality. Since the publication of the book ten years ago, more important new data have been published in this segment than in perhaps any other of the areas covered by the Selling-Ferraro textbook. It is a pity that this textbook of "psychodietetics," a term coined by Fritz,[28] does not give bibliographical references to the literature it cites. Fritz[29] prepared a classified bibliography of the older, predominantly clinical, rather than experimental, studies in this field.

Food Selection and Appreciation

On the psychophysiological side, the chemical senses of gustation and olfaction have been studied intensively, both in their fundamental aspects (Ref. 31, pp. 270–323),[60,61] and in reference to the flavor of foods.[20,51,54] The odor problems in science and industry (including the problem of controlling odors in processing protein foods)[22] were discussed at a recent conference, with A. R. Behnke as chairman. Procedures for the evaluation of food acceptability, applied in the Quartermaster Food and Container Institute (Chicago), were described by Dove.[23] The problems of creating markets for new food products, and of loosening old and stabilizing new brand loyalties have very important economic implications and represent an important facet of the art and science of advertising.

There is a large literature on thirst and hunger, as bodily needs for water and food

(or foods) (Ref. 56, pp. 382–401). The mechanisms involved in the regulation of food intake were considered recently by Mayer[50] in reference to the etiology of obesity and with emphasis on the author's "glucostatic" theory. The literature on appetite, palatability of foods, and food habits, studied from the point of view of experimental psychology, was critically reviewed by Young.[78] Food habits became the object of intensive study in America during the Second World War and the Committee on Food Habits, National Research Council, prepared a trail-blazing report on the problem of changing food habits,[18] and later prepared a manual, with an extensive bibliography (682 items), on the methodology of food habits research.[19] The authors visualized a wide audience, including those concerned with the study of foodways within the framework of cultural anthropology; those responsible for large-scale social surveys in which the food habits of the population will be one of the items covered; and those whose professional duties include the application of available knowledge on food habits and provide opportunities for further research in this field, such as specialists in child care and nursery school education, family life, public welfare, mental hygiene, and psychosomatic medicine.

Experiments on Diet and Behavior

(a) *Calories:*

In the remaining part of this presentation, our attention will be limited to the impact of nutrition on behavior, with special reference to calories, vitamins, and amino acids. It may be expected that the lack of any essential nutrient would produce, in time, profound deterioration in one or more aspects of performance and personality (cf. the association of low mentality and congenital deafness with endemic, iodine-deficiency goiter and cretinism).

The nutritional conditions that have been most intensively studied are inanition and the partial restriction of calories. From the dietary point of view, the quantity of food could be readily controlled long before the advances in the knowledge of vitamin and amino acid composition of foods, and before it was practically possible to make up rations adequate in all but one nutrient and to supplement the existing diet with purified or synthetic components.[2]

It may be noted that an extensive study on the psychophysiology of prolonged fast was reported by Langfeld as early as 1914.[48] The subject was a lawyer from Malta, Agostino Levanzin, who came to Boston to undergo a prolonged fast at the Nutrition Laboratory of the Carnegie Institution of Washington. Langfeld's task was to supplement physiological measurements by psychological tests. Both sensory (tactual space threshold, visual acuity), psychomotor (tapping, strength), and intellective functions (memory for words and digits, word association, letter cancellation) were examined. There was a surprising absence of definite deterioration, and in some tests there was an apparent improvement, due—at least in part—to learning. Control measurements were done only on two days before the beginning of the fast, which would not be sufficient to reach a learning plateau in many of the tests used in the study.

The Boston laboratory of the Carnegie Institution of Washington, with Walter Miles in charge of the psychological measurements, undertook in 1917–18 a fairly comprehensive study of the body's response to prolonged caloric restriction.[8] The study was prompted by the existence of severe food shortages in Europe during the latter part of the First World War, but the dietary rehabilitation was not systematically studied. The loss of body weight was small (about 10 per cent of the initial value).

A much more severe test of the effects of caloric deprivation on performance and personality was made in the Minnesota starvation-rehabilitation experiment carried out in 1944 to 1946. In the detailed report of this study,[44] the data obtained at Minnesota are presented against the background of experimental investigations reported in the literature and the wealth of clinical and field observations on undernutrition and famine, including those made in Holland in 1945.

Several papers were devoted to special problems.[13,14,26,27,45,69]

While systematic, quantitative studies on the physical aspects of semistarvation and of the recovery phase are few in number, no such studies have been previously reported on the psychological aspects. The clinical and field reports on behavior are based largely on qualitative observations. Obviously, standardized psychometric or sociometric methods are not readily applicable under conditions of semistarvation and famine. In the Minnesota study, a wide variety of techniques was used for the assessment of the effects of prolonged food restriction (involving an average intake of less than 1600 cal. per man per day for six months, with the resulting loss of 25 per cent of body weight). The outstanding features were a large loss of strength, a marked loss of endurance in physical work, with small changes in speed and co-ordination, no or negligible impairment in sensory functions and intellective performance, and marked changes in the feeling of well-being and in personality, with consistent trends in the direction of "psychoneurosis." In general, the amount of recovery tended to parallel the caloric value of the diets on which the different subgroups were maintained for the first eight weeks of dietary rehabilitation. Follow-up studies on twenty men, made 33 weeks after the end of the starvation diet, indicated a complete recovery, as judged by the scores on the Minnesota Multiphasic Personality Inventory and strength of grip.

(*b*) *Vitamins:*

In the biology of mental health an important role is played by the vitamins of the B complex, notably thiamine. At the meeting of the Association for Research in Nervous and Mental Disease,[7] devoted to considerations of the effects of nutritional deficiencies on personality, vitamin B complex received a large amount of attention. It was readily evident, however, that the experimental study of behavioral alterations was much less advanced than the medical (clinical) and biochemical research on the effects of B-vitamin restriction. At that time, studies using standardized, quantitative techniques for the investigation of different aspects of performance capacity and personality were severely limited in number and coverage. The study by O'Shea and Elsom[59] on mental changes in B-complex deficiency should be noted among pioneering efforts in this direction. The literature on the role of vitamin deficiencies in nervous and mental disorders was reviewed extensively by Hardwick.[35]

Wilder[77] summarized the early experimental studies carried out in Rochester, Minnesota, between 1939 and 1942, and surveyed the more recent literature on experimental induction of psychoneuroses through restriction of the thiamine content of the diet. The subjects in the Rochester studies were young to middle-aged female inmates of the local state Mental Hospital, regarded by the psychiatric staff as "recovered" from the acute phase of their mental illness. The complications of interpretation of the results, introduced by the initial psychiatric abnormality of the patients, were balanced out, in part, by a long prerestriction period, allowing adequate "control" observations, and a rigorous control of diet and environment. The periods of thiamine deficiency were followed by an after-period of sufficient length to allow a complete recovery from the abnormalities which developed during the period of thiamine restriction.

In one series of observations, the thiamine content of the diet was reduced to 450 μg. per day. In the control period, the ten subjects were fairly well adjusted, industrious, and efficient in their daily tasks. "Some weeks after the thiamine was restricted they were neglecting their work and followed instructions poorly. They became forgetful, irritable, quarrelsome, apathetic, confused, depressed, restless, and anxious. In the end, but not until after many weeks, symptoms and minimal signs of peripheral neuropathy developed in some but not in all subjects. These disappeared gradually as the allowance of thiamine was increased" (Ref. 77, p. 531). No measurement of the degree of psychological abnormality was attempted in these studies.

The effects of the restriction and total deficiency of thiamine in eight previously normal young men have been examined in the Laboratory of Physiological Hygiene (overall picture, including biochemical and physiological data;[43] personality;[12] intellective functions;[33] motor performance[11]). The experiment consisted of four periods: standardization (41 days); partial restriction (161 days) with intakes of 0.18 mg. thiamine, 0.29 mg. riboflavin, and 3.7 mg. niacin per 1000 calories; period of acute deficiency of these vitamins (23 days), with only a trace of these vitamins in the diet; and a 10-day period of thiamine supplementation.

Self-ratings and intersubject (man-by-man) ratings gave no evidence of change in the well-being and emotional adjustment during the long period of partial restriction, but indicated a consistent and striking deterioration of this aspect of overall "fitness" during the acute deficiency. During this period the men were fed a synthetic diet, largely composed of cornstarch, sugar, vegetable shortening, and purified casein, to which minerals and vitamins were added to produce a balanced diet, except for the B-complex vitamins. Anorexia, and later nausea and vomiting began at 8 to 14 days and progressed to an almost complete inability to eat in another 10 days. Supplementation of the diet by thiamine alone produced return of appetite, a rapid recovery in well-being, and regression of subjective symptoms as measured by the ratings.

The Minnesota Multiphasic Personality inventory also gave no evidence of deterioration during the period of partial restriction. During the acute deficiency, dramatic changes were obtained in the mean scores of the experimental subjects on the three psychoneurotic scales—Hypochondriasis (from 46 to 80), Depression (from 58 to 87), and Hysteria (from 55 to 83); "normal" mean = 50, 1 SD = 10. It appears that the deficient men did not develop a pattern of personality deterioration specific to the vitamin B-complex deficiency, but became indistinguishable from many severely neurotic patients.

In the period of partial restriction, no evidence of intellective deterioration was obtained using two tests of general intelligence and Porteus mazes. During acute deprivation of vitamins of the B-complex, some impairment in a battery of six short tests of intellective functions[34] was noted, especially in the tests in which *speed* was an essential factor. However, only in the test of ability to visualize objects in space ("Flags") did the difference between the deficient and supplemented subjects reach a 5 per cent level of statistical significance.

In the period of partial restriction, there was no actual deterioration in any of the psychomotor measurements. In two tests (speed of tapping and speed of co-ordinated hand-and-arm movements) out of the seven tests the mean scores showed somewhat larger practice increments in the supplemented subjects (supplements of 1 mg. thiamine, 1 mg. riboflavin, 10 mg. niacin daily, at a caloric expenditure of about 3300 cal.) than in the restricted group. The difference was statistically significant at the 5 per cent level. In the period of acute deficiency there was evidence of a marked deterioration of psychomotor performance. It was already present by the 11th day of maintenance on a diet essentially free of vitamins of the B-complex, and was one of the early symptoms of deficiency. Decreases in strength and speed of tapping were small and did not reach the level of statistical significance. Significant (5 per cent level) deterioration was obtained in the two tests of reaction time, involving the movement of the toe and of the whole body, and in the speed of hand-and-arm movements. Highly significant (1 per cent) deterioration was noted in the test of eye-hand co-ordination. Psychomotor performance tended to return to a normal level after 10 days of liberal supplementation of the diet with thiamine (10 mg.; later 5 mg. daily).

In children and some other groups, no dietary restrictions can be imposed experimentally, for humanitarian reasons. These groups are still amenable to, and in some instances eminently suitable for, dietary manipulations involving supplementation of the existing diet with a particular nutrient, if a case may be made theoretically that the

subjects could benefit from such a dietary treatment. Two studies on the effect of adding 2 mg. of thiamine to an "average" diet, providing about 0.9 mg. thiamine per child per day, were carried out by Harrell[36,37] (cf. *Nutrition Reviews* 4: 343–345, 1946; 7: 220–222, 1949). The author concludes that the effects of thiamine supplementation may not be observable over a short period, but that the cumulative effects of a superior dietary regimen throughout a lifetime may spell the difference between alert, successful living and marginal effectiveness (Ref. 37, p. 100). While the general validity of this thesis cannot be denied, on repeating the study with identical twins as subjects,[9,68] followed over a period of nine months, no statistically significant differences were obtained, although there was some tendency toward slightly superior gains in the supplemented group.

The overwhelming majority of behavioral manifestations of nutritional deficiencies are non-specific. The neurasthenic syndrome, present in vitamin B-complex (thiamine) deficiencies may result from a multiplicity of non-nutritional causes. Undue fatigue, restlessness and irritability, vague aches and pains, fearfulness and forgetfulness, sensitivity to noise, and lack of appetite should not be readily assigned to faulty nutrition and treated, without regard for dietary history, with vitamin pills.

A specific sensory impairment is associated with a deficiency of vitamin A. While there has been a good deal of variability in the reported onset and severity of the symptom (cf. *Nutrition Reviews* 7: 234–238, 1949), a rise in the light threshold of the dark-adapted eye has been repeatedly observed in men (and rats[55]) maintained on diets deficient in vitamin A. A meticulous, large-scale study on the effects of vitamin A deficiency was carried out under the sponsorship of the British Medical Research Council.[38] Twenty young men and three women, who volunteered for the study as conscientious objectors, served as subjects. Sixteen of them lived on a diet virtually free of vitamin A and extremely low in carotene from 6 to 25 months. Measurements of dark adaptation were made frequently. A marked deterioration of dark adaptation (increase in the final light threshold) was observed in only 3 out of 16 experimental subjects, after 10, 12, and 20 months of maintenance on the deficient diet. There was also an increase in the cone-rod transition time (i.e., the length of time, from the beginning of the dark adaptation period, at which rods replace cones as mediators of vision) and in the total time needed for reaching the maximal light sensitivity. These results, which should not be generalized without caution, indicate that in adults of average ("normal") nutritional status at the outset, deficiency of vitamin A can be induced less rapidly and less readily than had been thought before these data became available.

(c) *Amino acids:*

One of the problems that has aroused a good deal of interest and brought forth a whole series of experimental investigations concerned the mental effects of glutamic acid (cf. *Nutrition Reviews* 9: 113–117, 1951; 11: 201–204, 1953). Clinical research on the potential effects of glutamic acid on intelligence was stimulated by the incidental observations of increased alertness during the treatment of epilepsy.[62] Results of the early studies on the use of glutamic acid supplements in mentally defective children seemed encouraging.[80] Later and better controlled investigation[24,49,52,53,79] in general indicated a lack of statistically significant beneficial effect of supplementary feeding of glutamic acid on measured intelligence in mentally retarded individuals. Positive results were reported by Albert, Hoch, and Waelsch[1] in children with very low mental ability (IQ from 22 to 60) and some improvements were noted by Foale[25] in children receiving 9 Gm. of glutamic acid during the first four months and 12 Gm. during the subsequent six months. The supplement was given in a form of tablets. Apparently no placebos were given to the control group. The initial and final mean IQ values, obtained by equivalent forms of Cattell's test of intelligence, were 62.1 and 65.3 in the supplemented, 64.1 and 64.2 in the control group. In the test of "practical

ability," the gains were almost identical in the two groups. An improvement in the general adjustment of a number of the experimental subjects was reported, in contrast to the absence of such changes in the control group. Methodologically, the apparent absence of placebos is a weakness of the study and may have affected differentially the overt behavior of the two groups.

Industrial Nutrition

In addition to laboratory investigations on performance capacity, a limited amount of controlled investigations has been carried out in industry. In the United States, the two principal problems were the effectiveness of vitamin supplementation (cf. *Nutrition Reviews* 8: 312–317 1950) and the between-meal-feeding (cf. *Nutrition Reviews* 8: 167–170, 1950). Recent literature on the influence of nutrition on work performance was summarized by Simonson[71] and Hutchinson and Krehl.[40] Selected aspects of German experiences, made during and following the Second World War, on dietary intake and performance were reviewed by Kraut;[47] some of these studies were critically evaluated in *Nutrition Reviews* 9: 340–342, 1951.

The broad field of industrial nutrition has been surveyed systematically by Pyke.[63] The author pointed out[64] that although the literature on the energy requirements is considerable, much of it does not apply to modern industrial work and is of limited value to those who are responsible for the task of designing meals for industrial workers.

A review of meal habits and their effects on performance was prepared by Hutchinson.[39] He was particularly concerned with in-between-meals feeding, stressing certain advantages in having a large number of small meals at short intervals (rather than a smaller number of large meals at long intervals). Such a dietary regimen apparently promotes alertness and general well-being, while large meals generally result in postprandial drowsiness and disinclination for mental effort and physical activity. The new material provided by the author consists of a graph representing a "typical" daily production curve, with a rise in output during the early part of the morning and the afternoon work period, and a "fatigue" decrement in the later part of each period. Of particular interest are the differences in the afternoon output curves following a heavier and a light midday meal. While after a light snack the production curve rises fairly steeply, a full meal is associated with a "flattening" in the early production curve (a slower rise). If numbers could be attached to these observations, the information would not be without interest. Unfortunately, no details are available on the type of industrial operations to which the graph refers, the number of workers involved, or the actual size of the meals. The graph has been prepared on the basis of descriptive information received by the author from unidentified sources. Thus, scientifically, hardly any progress has been made beyond the common-sense observation that ingestion of a large meal is frequently associated with a feeling of drowsiness, some deterioration of co-ordination, with disinclination for mental or physical effort.

The practice of between-meal feeding is generally considered as a positive factor in production efficiency. Jung[42] regards the mid-morning snack as especially important when the work starts early in the morning and the actual period since breakfast is lengthened by a substantial amount of time needed for traveling from home to work. However, in some situations the interruption of work requires a new "warm-up," with slowly rising output, and the practice of mid-morning snacks is of questionable merit, if evaluated on the basis of output alone. Again, this important point is not documented by actual data.

Jung[42] stressed that in intellectual work the mood plays an important role, and that it can be affected, in some measure, by the diet. He believes, specifically, that meat has a stimulating effect on many people. All these points are unquestionably of practical importance. Unfortunately, scientific investigations of these matters are very meager in comparison with the wealth of the medico-culinary lore. Even as far as muscular work is concerned, the literature on the effect of variations in the

composition of the diet is full of inconclusive and contradictory statements.

Potential Applications in Clinical and Field Studies

The problems discussed so far have been studied, principally, under carefully controlled laboratory conditions. A limited amount of controlled research has been carried out in industry. The extension of the application of psychometric techniques to the clinic and to the field represents a real challenge. Thus, while in pellagra the "dementia" (together with dermatitis and diarrhea) has long been recognized as a classical symptom, there is a dearth of investigations of the psychological aspects of the disease using modern methods for the study of personality.

It may be that those parts of the world in which serious food deficiencies are widespread represent the most fruitful and most important field for a systematic study of the impact of nutrition on psyche. In kwashiorkor—a disease associated with protein deficiency in young children and rampant in some parts of Africa, Asia, and the Americas—the presence of mental apathy, combined with peevishness when the child is disturbed, has been cited as one of the outstanding characteristics.[41] Clark[17] considers the mental changes a consistent and important feature of kwashiorkor. Davies (Ref. 21, p. 110) noted that the mental changes are unquestionably a part of the disease, do not respond to any specific therapy, and disappear on general clinical improvement. He suggests that they are directly related to protein deficiency and the associated general enzymic upset. Carothers[16] believes it unlikely that the impairment of mental development at two to four years, associated with severe protein deficiency, will be wholly remedied in later years. As the matter stands now, we lack data necessary to confirm or to refute this hypothesis.

The exploration of intellective growth in different populations requires non-verbal tests. Such a test, still not universal enough in content, and more "culture bound" than is desirable, has been recently developed and described by Gille.[32] The test, designed for group administration and applied to some 100,000 French children, has been used also with natives of French West Africa. The variations in the mode of life, envisaged as one of the topics to be studied by this test, include importantly the nutritional factors.

A study of psychological changes associated with kwashiorkor was initiated by a grant from the Mental Health Section of W.H.O. Performance on the individually administered battery of tests, which are largely manipulative, and which have been standardized by Gesell for the study of the mental growth of the preschool child, is used for the evaluation of mental capacity.[30] In addition to the psychometric studies, attention is being paid to the socio-psychological setting in which the disease develops, especially to the behavior of the mother.

While speculations abound about the role of food in national differences in temperament, empirically founded knowledge in this area is negligible. Primitive societies, of the same genetic background and living in the same climate but subsisting on different diets, provide useful "natural laboratories" for testing the hypothesis concerning diet and the psychological make-up. In Northern Rhodesia, Richards and Widdowson[60a] observed that the fish-eating Bisa exhibited greater vitality, endurance, and ability to concentrate than the Bemba, in whose diet cereals (millet) played a dominant role.

Nicol,[58] in his study of tribal nutrition and health in Nigeria, reported that, on clinical examination, physical fitness and mental alertness were judged satisfactory in about 70 per cent of the trader group and the Ijaw fishermen, while only half as many Isoko farmers appeared fit and the majority of them were apathetic during the examination. Considerations of their general appearance, behavior, and dietary intake led the author to believe that the mental apathy and irritability of the Isoko farmers was causally related to a prolonged deficiency of B-complex vitamins. A more detailed diagnostic study and the evaluation of the effects of dietary treatments will profit from the application of more sophisticated techniques for the description of

mental functioning and of personality. Nicol's study was limited primarily to a qualitative clinical evaluation, but records of the local police and the courts, providing evidence on the rages and tantrums from which the members of the Isoko tribe suffer from time to time, were also considered.

Animal Studies

So far we have dealt with man. Dr. Paule Aschkenasy-Lelu, who has written reviews of the literature dealing with the effects of nutritional deficiencies on behavior,[4] supplementation of the diet with certain nutrients, particularly thiamine and glutamic acid,[3] and nutrition in patients with mental disorders,[6] has also carried out experimental studies[5] on the spontaneous food intake of the rat. While she accepts the general thesis that metabolic factors play an essential role in regulating food intake, she has been unable to demonstrate the presence of a specific "hunger" for protein.

The study of nutrition and animal behavior is an interstitial area, depending for effective development on the combination of competence in biochemistry and familiarity with methods for the study of animal behavior. As far as I know, there is not a single laboratory devoted to a systematic study of this area; textbooks of nutrition have largely neglected studies on animal behavior, which are scattered in a wide variety of publication media, most of them not readily accessible to the nutritionist. The Cumulative Index to the *Journal of Nutrition* for volumes 16 to 36, 1938 to 1948, carries no entry for "behavior," "performance," "activity," or "intelligence" studied in animals in reference to nutrition. In the period covered by the index, eight animal studies were published in the *Journal* dealing with self-selection of diets and the appetite for different nutrients.

The best reference is Munn's *Handbook of Psychological Research on the Rat*[57] which reviews the fairly large literature on the hunger cycle and the eating behavior, on nutrition and the general activity (as measured by the use of revolving drums and tambour-mounted cages), diet and sexual behavior, dark adaptation in the rat and avitaminosis A, nutrition and learning, and dietary factors in relation to the susceptibility to audiogenic seizures. Reed (Ref. 65, p. 397) considered nutritional factors in his review of literature on spontaneous activity of animals.

The importance of special senses (vision, smell) to the grazing sheep has been investigated recently at the Rowett Research Institute.[72,73] Tribe[73] wrote a detailed review of the behavior of the grazing animal, taking into account taste, smell, and sight. He concludes that available knowledge is not sufficient for a satisfactory theoretical interpretation of appetitive behavior. In addition to individual factors, the social facilitation in grazing behavior, induced by the presence of a group of sheep receiving no food supplement (one pound of linseed cake meal and crushed oats), was studied.[74] When the two groups grazed separately, the supplemented animals spent more time resting than the unsupplemented group. When the two groups grazed together, the proportion of grazing and resting time was similar and approximated the behavior of the unsupplemented group when grazing alone.

An ingenious technique for the measurement of endurance was applied by Kniazuk and Molitor[46] in their study of thiamine deficiency. By attaching different weights to swimming rats, motivated to perform by fear of drowning, the authors were able to vary the work load in a simple way. This approach is preferable to forced performance to exhaustion on a motor-driven treadmill, because the possibility of a mechanical injury to the feet of the animal is completely eliminated.

Recent literature on animal behavior in vitamin B-complex deficiencies has been summarized in reference to maze learning (*Nutrition Reviews* 10: 235–238, 1952) and to the establishment of conditioned responses (*Nutrition Reviews* 10: 273–276, 1952). Qualitative observations of changes in behavior are useful raw data, pointing out the importance of nutritional factors. Thus Rinehart, Greenberg, and Ginzton[67] reported that removal of thiamine from the diet resulted within about

two weeks in diminished food consumption and weight loss. In time the animals became weak, apathetic, and inactive. They developed muscular inco-ordination, drooping of the upper eyelids, and tremors.

While these observations leave no doubt about the effects of the thiamine-deficient diets, application of quantitative procedures, developed for research on animal behavior, are needed for the study of the relationship between the quantitative parameters of the deprivation (its duration and severity) and the sensory and motor functions.

In the early research on vitamins of the B complex and learned behavior, the animals were trained to run in mazes of the alley-type. Food, serving as incentive, was placed at the end of the maze. Since loss of appetite is one of the early symptoms of deficiency of the B-complex vitamins, especially thiamine, a poor performance of the deficient animals could be due simply to a change in motivation, without a deterioration in learning capacity. For this reason, the use of escape from water, rather than finding food, is preferable as a motive for performance. It has been used, e.g., by Whitley, O'Dell and Hogan[76] in their study of folacin deficiency. The deficient animals showed statistically significant inferiority in their maze-learning ability.

Learning to run a maze may be referred to, following Allport, as *efferent* learning, involving alteration in the response to a given set of stimuli. Acquisition of conditioned responses, on the other hand, represents *afferent* learning, consisting in the extension of the range of effective stimuli. On the basis of a limited amount of available experimental data, it appears that conditioned responses may serve as highly sensitive indicators of nutritionally induced impairment of the central nervous system.

It was noted above that water mazes are preferable to dry-land mazes. However, swimming involves large musculature activity, and muscular weakness could simulate impairment of the learning ability. The use of conditioned responses, such as the closure of the eyelid resulting from applying a puff of air to the cornea, simultaneously with the presentation of a light stimulus,[10] serves to eliminate the interference of variable motivational factors and to reduce the importance of muscular weakness. Establishment of conditioned responses (e.g., closure of the eyelid at the appearance of light alone) is a useful tool for the evaluation of the animal's ability to develop new stimulus-response associations. Strangely enough, this technique has largely been neglected in research on nutrition and learning, where the reliance has been placed almost exclusively on maze learning.

While the limitations of animal studies in general (and of the researches on behavior in particular) cannot be overlooked, it appears that the techniques for the study of animal behavior have not been fully utilized in the interest of the science of nutrition.

Summary and Conclusions

Our attention has been focused on experimental investigations of the impact of food on behavior in man, with a brief reference to animal studies. Selected illustrative studies were reviewed, with particular reference to the effects of the variations in the intake of calories and two classes of special nutrients, the vitamins and the amino acids, specifically glutamic acid.

Beneficial psychological effects of the supplementation of a good "average" diet of "normal" individuals with any nutrient still remains to be demonstrated satisfactorily. At the same time, there is no doubt that dietary deficiencies affect profoundly man's willingness and capacity for work, and alter his personality. As a rule, these alterations in attitude and behavior simulate psychoneurosis, although in some instances faulty diet may bring about psychotic changes, associated with profound disorders of the central nervous system (some patients with pellagra, encephalopathy in acute nicotinic acid deficiency, the Wernicke syndrome in the alcoholic subject). In the United States, the vitamin enrichment of flour and bread, begun in 1941, seems to have markedly reduced the incidence of these symptoms of avitaminosis (Ref. 77, p. 536). Wilder[77] noted also the decline of severe mental disturbances, on adding thiamine to

the glucose administered in cases of pernicious vomiting of pregnancy or after some major surgical operations.

It should be noted that in human experimental studies, as a rule, the subjects have been young men, selected for freedom from disease and living in comfortable and hygienic surroundings. They were charged with the responsibility of performing as well as they could and, with their high motivation (varying in the guiding values in the case of medical students, soldiers, and conscientious objectors), in the overwhelming majority they have lived up to the high expectations. The deterioration in the measured aspects of performance capacity and personality has certainly been minimal, and careful judgment must be exercised in extrapolating the results.

In industry, agriculture, and the majority of military operations, brute force is being steadily replaced by that of the machine. Consequently, in testing man's "fitness" in reference to the dietary intake, the importance of psychological components of fitness has been steadily increasing.

Motivation, a crucial factor in all achievement, is particularly readily affected by improper nutrition. Apathy and psychoneurosis, experimentally induced in the laboratory, have their counterpart in the behavior and attitudes of malnourished populations. Further extension of the knowledge of "psychodietetics" is one of the challenging tasks for the science of nutrition. At the same time, a vigorous and intelligent application of available information would raise significantly the physical and mental health and productivity of the inhabitants of the underdeveloped and underfed areas of the world.

REFERENCES

1. Albert, K., Hoch, P., and Waelsch, H.: Glutamic acid and mental deficiency. *J. Nerv. & Ment. Dis.* 114: 471, 1951.
2. Anderson, M., Mickelsen, O., and Keys, A.: Diets and food items for the experimental production of thiamin deficiency in man. *J. Am. Dietet. A.* 22: 1, 1946.
3. Aschkenasy-Lelu, P.: Le doping alimentaire du psychisme. *Ann. Nutr. Alim.* 3: 109, 1949.
4. Aschkenasy-Lelu, P.: Le retentissement de la nutrition sur les phénomènes psychiques. 1. Alimentation et conduite. *Encéphale* 40: 341, 1951.
5. Aschkenasy-Lelu, P.: Ingestion alimentaire en fonction de la composition du régime. Application à la régulation du besoin azoté chez le rat. *Ann. Nutr. Alim.* 5: 453, 1951.
6. Aschkenasy-Lelu, P.: Le retentissement de la nutrition sur les phénomènes psychiques. 2. Nutrition et psychisme. *Encéphale* 41: 45, 1952.
7. Association for Research in Nervous and Mental Disease: *The Role of Nutritional Deficiency in Nervous and Mental Disease.* Williams and Wilkins, Baltimore. (Res. Publ. Assn. Nerv. Mental Dis., vol. 22, 1943).
8. Benedict, F. G., Miles, W. R., Roth, P., and Smith, H. M.: Human vitality and efficiency under prolonged restricted diet. *Carnegie Inst. Washington Publ.* No. 280, 1919.
9. Bernhardt, K. S., Northway, M. L., and Tatham, C. M.: The effect of added thiamine on intelligence and learning with identical twins. *Canad. J. Psychol.* 2: 58, 1948.
10. Biel, W. C., and Wickens, D. D.: The effect of vitamin B_1 deficiency on the conditioning of eyelid responses in the rat. *J. Comp. Psychol.* 32: 329, 1941.
11. Brožek, J., Guetzkow, H., Mickelsen, O., and Keys, A.: Motor performance of normal young men maintained on restricted intakes of vitamin B complex. *J. Appl. Psychol.* 30: 359, 1946.
12. Brožek, J., Guetzkow, H., and Keys, A.: A study of personality of normal young men maintained on restricted intakes of vitamins of the B complex. *Psychosomat. Med.* 8: 98, 1946.
13. Brožek, J., and Erickson, N. K.: Item analysis of the psychoneurotic scales on the Minnesota Multiphasic Personality Inventory in experimental semistarvation. *J. Consult. Psychol.* 12: 403, 1948.
14. Brožek, J., Guetzkow, H., Baldwin, M. V., and Cranston, R.: A quantitative study of perception and association in experimental semistarvation. *J. Personality* 19: 245, 1951.
15. Brožek, J.: *Performance and Nutritional Status. Methods for Evaluation of Nutritional Adequacy and Status: A Symposium.* (In press, 1955.)
16. Carothers, J. C.: The African mind in health and disease: A study in ethnopsychiatry. *World Health Org. Monogr.* No. 17 Geneva, 1953.
17. Clark, M.: Kwashiorkor. *East Afr. Med. J.* 28: 229, 236, 1951.
18. Committee on Food Habits: The problem of changing food habits. *Bull. Nat. Res. Council* No. 108, Washington, D. C., 1943.

19. Committee on Food Habits: Manual for the study of food habits. *Bull. Nat. Res. Council* No. 111, Washington, D. C., 1945.
20. Crocker, E. C.: *Flavor.* McGraw-Hill Co., New York, 1945.
21. Davies, J. N. P.: Nutrition and nutritional diseases. *Ann. Rev. Med.* 3: 99, 1952.
22. Dorn, H. W.: The problems of controlling odors in processing protein foods. *Ann. New York Acad. Sc.* 58: 27, 1954.
23. Dove, W. F.: Food acceptability—its determination and evaluation. *Food Technology* 1: 39, 1947.
24. Ellson, D. G., Fuller, P. R., and Urmston, R.: The influence of glutamic acid on test performance. *Science* 112: 248, 1950.
25. Foale, M.: The treatment of mental defectives with glutamic acid. *J. Ment. Sc.* 98: 483, 1952.
26. Franklin, J. C., Schiele, B., Brožek, J., and Keys, A.: Observations on human behavior in experimental semistarvation and rehabilitation. *J. Clin. Psychol.* 4: 28, 1948.
27. Franklin, J. C., and Brožek, J.: The Rosenzweig P-F test as a measure of frustration response in semistarvation. *J. Consult. Psychol.* 13: 293, 1949.
28. Fritz, M. F.: The field of psychodietetics. *Psychol. Clin.* 22: 181, 1933.
29. Fritz, M. F.: A classified bibliography on psychodietetics. *Psychol. Monogr.* No. 206, 1934.
30. Geber, M., and Dean, R. F. A.: Psychological factors as adjuvants in the aetiology of kwashiorkor. (To be published, 1955.)
31. Geldard, F. A.: *The Human Senses.* John Wiley & Sons, New York, 1953.
32. Gille, R.: Les documents de base: Le questionnaire et le test mosaïque. *Institut National d'Études Démographiques, Travaux et Documents, Cahier* No. 13, 1950, pp. 71–94.
33. Guetzkow, H., and Brožek, J.: Intellectual functions with restricted intakes of B-complex vitamins. *Am. J. Psychol.* 59: 358, 1946.
34. Guetzkow, H., and Brožek, J.: Intellective tests for longitudinal experiments on adults. *Am. J. Psychol.* 60: 350, 1947.
35. Hardwick, S. W.: Vitamin deficiency in nervous and mental disorder. *Recent Progress in Psychiatry* 2: 111, 1950.
36. Harrell, R. F.: Effect of added thiamine on learning. *Columbia Univ. Teach. Coll. Contrib. Educ.* No. 877, 1943.
37. Harrell, R. F.: Further effects of added thiamine on learning and other processes. *Columbia Univ. Teach. Coll. Contrib. Educ.* No. 928, 1947.
38. Hume, E. M., and Krebs, H. A.: Vitamin A requirements of human adults: An experimental study on vitamin A deprivation in man. *Med. Res. Council Spec. Rep. Ser.* No. 264, 1949.
39. Hutchinson, R. C.: Meal habits and their effects on performance. *Nutr. Abstr. & Rev.* 22: 283, 1952.
40. Hutchinson, R. C., and Krehl, W. A.: The effect of food intake on performance. *Borden's Rev. Nutr. Res.* 15: 33, 1954.
41. Joint FAO/WHO Expert Committee on Nutrition: Third report. *WHO Tech. Rep. Ser.* No. 72, 1953.
42. Jung, A.: Ernährung und Leistungsfähigkeit. *Gesundheit & Wohlfahrt* (Zürich) 31: 12, 1951.
43. Keys, A., Henschel, A., Taylor, H. L., Mickelsen, O., and Brožek, J.: Experimental studies on man with a restricted intake of the B vitamins. *Am. J. Physiol.* 144: 5, 1945.
44. Keys, A., Brožek, J., Henschel, A., Mickelsen, O., and Taylor, H. L.: *The Biology of Human Starvation.* Univ. of Minn. Press, Minneapolis, Minn., 1950.
45. Kjenaas, N. K., and Brožek, J.: Personality in experimental semistarvation: A Rorschach study. *Psychosom. Med.* 14: 115, 1952.
46. Kniazuk, M., and Molitor, H.: The influence of thiamin-deficiency on work performance in rats. *J. Pharmacol. & Exper. Therap.* 80: 362, 1944.
47. Kraut, H.: Leistungsfähigkeit und Ernährung. *Experientia—Supplementum* I: 54, 1953.
48. Langfeld, H. S.: On the psychophysiology of a prolonged fast. *Psychol. Monogr.* No. 71, 1914.
49. Loeb, H. G., and Tuddenham, R. D.: Does glutamic acid administration influence mental function? *Pediatrics* 6: 72, 1950.
50. Mayer, J.: Genetic, traumatic and environmental factors in the etiology of obesity. *Physiol Rev.* 33: 472, 1953.
51. McCord, C. P., and Witheridge, W. N.: *Odors: Physiology and Control.* McGraw-Hill Co., New York, 1949.
52. McCulloch, T. L.: The effect of glutamic acid feeding on cognitive abilities of institutionalized mental defectives. *Am. J. Ment. Defic.* 55: 117, 1950.
53. Milliken, J. R., and Standen, J. L.: An investigation into the effects of glutamic acid on human intelligence. *J. Neurol., Neurosurg. & Psychiat.* 14: 47, 1951.
54. Moncrief, R. W.: *The Chemical Senses* (2nd ed.). L. Hill, London, 1951.
55. Morgan, C. T.: Studies in vision. II. Dark adaptation in normal and A-avitaminotic rats. *J. Genet. Psychol.* 60: 109, 1942.
56. Morgan, C. T., and Stellar, E.: *Physiological Psychology* (2nd ed.). McGraw-Hill Co., New York, 1950.
57. Munn, N. L.: *Handbook of Psychological Research on the Rat: An Introduction to Animal Psychology.* Houghton Mifflin Co., Boston, 1950.

58. Nicol, B. M.: Tribal nutrition and health in Nigeria. J. Clin. Nutrition 1: 364, 1953.
59. O'Shea, H. E., and Elsom, K. O.: Studies of the B vitamins in the human subject: IV. Mental changes in experimental deficiency. *Am. J. Med. Sc.* 12: 388, 1950.
60. Patton, H. D.: Physiology of smell and taste. *Ann. Rev. Physiol.* 12: 469, 1950.
61. Pfaffmann, C.: Taste and smell. Ch. 29, pp. 1143–1171, in *Handbook of Experimental Psychology*, S. S. Stevens, ed., John Wiley & Sons, New York, 1951.
62. Price, J. C., Waelsch, H., and Putnam, T. J.: Glutamic acid hydrochloride in the treatment of petit mal and psychomotor seizures. *J. A. M. A.* 122: 1153, 1943.
63. Pyke, M.: *Industrial Nutrition.* MacDonald and Evans, London, 1950.
64. Pyke, M.: Industrial nutrition. *Nutrition Rev.* 9: 97, 1951.
65. Reed, J. D.: Spontaneous activity of animals. *Psychol. Bull.* 44: 393, 1947.
66. Richards, A. I.: *Hunger and Work in a Savage Tribe: A Functional Study of Nutrition Among the Southern Bantu.* The Free Press, Glencoe, Ill., 1948.
66a. Richards, A. I., and Widdowson, E. M.: A dietary study in Northeastern Rhodesia. *Africa* 9: 166, 1936.
67. Rinehart, J. F., Greenberg, L. D., and Ginzton, L. L.: Thiamin deficiency in the rhesus monkey. *Blood* 3: 1453, 1948.
68. Robertson, E. C., Tatham, C. M., Walker, N. F., and Weaver, M. R.: The effect of added thiamine on growth, vision, and learning using identical twins. *J. Nutrition* 34: 691, 1947.
69. Schiele, B. C., and Brožek, J.: "Experimental neurosis" resulting from semistarvation in man. *Psychosomat. Med.* 10: 31, 1948.
70. Selling, L. S., and Ferraro, M. A. S.: *The Psychology of Diet and Nutrition.* W. W. Norton, New York, 1945.
71. Simonson, E.: Influence of nutrition on work performance. *Nutr. Symp. Ser.* No. 3, 1951, pp. 72–114.
72. Tribe, D. E.: The importance of the sense of smell to the grazing sheep. *J. Agric. Sc.* 39: 209, 1950.
73. Tribe, D. E.: The behaviour of the grazing animal: A critical review of present knowledge. *J. Brit. Grassland Soc.* 5: 209, 1950.
74. Tribe, D. E.: Influence of pregnancy and social facilitation on the behaviour of the grazing sheep. *Nature* 166: 74, 1950.
75. Tribe, D. E., and Gordon, J. G.: The importance of colour vision to the grazing sheep. *J. Agric. Sc.* 39: 313, 1950.
76. Whitley, J. R., O'Dell, B. L., and Hogan, A. G.: Effect of diet on maze learning in second generation rats. Folic acid deficiency. *J. Nutrition* 45: 153, 1951.
77. Wilder, R. M.: Experimental induction of psychoneurosis through restriction of intake of thiamine. Ch. 31, pp. 531–538, in the *Biology of Mental Health and Disease.* P. B. Hoeber, New York, 1952.
78. Young, P. T.: Appetite, palatability and feeding habit: A critical review. *Psychol. Bull.* 45: 289, 1948.
79. Zabarenko, R. N., and Chambers, G. S.: An evaluation of glutamic acid in mental deficiency. *Am. J. Psychiat.* 108: 881, 1952.
80. Zimmerman, F. T., Burgemeister, B. B., and Putnam, T. J.: Effect of glutamic acid on mental functioning in children and adolescents. *Arch. Neurol. & Psychiat.* 56: 489, 1946.

Does a Low Intake of Calcium Cause or Promote the Development of Rickets?

By A. R. P. WALKER, M.SC., PH.D.*

THE PRESENT position regarding the requirement of calcium and the signs of its deficiency is far from satisfactory. This is evident from recent publications. Thus, Hegsted and co-workers[1,2] have noted that the recommended allowances of calcium are based largely on balance observations, the uncertainties of interpretation of which are well known. Goldsmith,[3] moreover, has reiterated Macy's contention of ten years ago, namely, that "neither the most satisfactory level of calcium intake nor the optimal retention of calcium at any physiologic age or stage of man's development is known."

A high intake of the element is often recommended on the grounds that a low intake causes or predisposes to the development of rickets.[4–10] But it is noteworthy that standard textbooks on pediatrics, in the main, do not appear to regard level of calcium intake of much significance.[11–13] Moreover, other authorities reject the implication of calcium deficiency in the etiology of the disease, maintaining that it does not occur provided adequate vitamin D be present (excluding vitamin D-resistant rickets).[11,14,15] In view, first, of the differences of opinion on the subject, and second, its relevance to recommended calcium allowances, it has been thought desirable to examine the literature and seek to learn to what extent calcium is involved in the etiology of the disease. The conclusion reached in this paper is that there is no specific evidence that a low calcium intake promotes, still less causes rickets, such findings thereby increasing misgivings on current views on calcium requirement and deficiency.

The first point to be appreciated is that there are a large number of factors which militate against a precise evaluation of the role of calcium intake in rickets.†

FACTORS WHICH MILITATE AGAINST SATISFACTORILY ASSESSING THE ROLE OF CALCIUM DEFICIENCY IN THE ETIOLOGY OF RICKETS

(*1*) *Uncertainties in Diagnosis*

There are four types of diagnostic criteria: clinical, radiological, histo-pathological, and chemical analysis of bone.

(a) *Clinical Assessment.* Among pediatricians, there is no dispute as to the signs of severe rickets. But the same unanimity of opinion is absent over mild rickets, the clinical signs of which Park[16] stated to be "notoriously deceiving and cannot be relied upon."

(b) *Radiological Assessment.* The previous statement likewise applies to diagnosis from roentgenograms. It must be stressed that the radiological picture associated with poorly mineralized bone can arise from many causes and, as Youmans[17] has pointed out, too often osteoporosis has been uncritically interpreted as the result of lack of calcium. Furthermore, the poor measure of agreement between clinical and radiological assessments of rickets deserves emphasis. In the British Pediatric Survey,[18] which was carried out in 1942 by a panel of specialist workers, the clinicians confirmed positive radiological opinion in less than half the cases of rickets;

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† Throughout this paper *clinical* rickets is in mind unless otherwise stated.

conversely, the radiologists confirmed positive clinical opinion in one out of twenty-five cases.

(c) *Histo-pathological Assessment.* Here, the assessment is based on microscopic examination of a section of the costo-chondral junction of the rib. Unfortunately, however, there are differences in grading adopted by different groups of workers. In the comprehensive studies of Follis and co-workers,[19,20] histologic rickets was stated to be present in over half of the large series of American children examined at necropsy, severe rickets being present in about two-thirds of the cases. Were the same standard of critical judgment used for non-American children (usually in a less privileged nutritional condition), the proportion graded as *free* from rickets would be very small indeed, in which case the condition could be regarded as of normal or even of physiological occurrence.

(d) *Chemical Analysis of Bone.* Very few studies on the composition of human bone in rickets have been carried out, as is evidenced by Hawk *et al.*[21] citing German studies exclusively, which were published over forty years ago. A recent investigation has been carried out by this Unit on the chemical composition and histological appearance of the fifth rib of South African Bantu infants, children, and adults (also European controls). In mild rickets, it was found that mineralization data (ash, calcium, and phosphorus) lay within the same range as for non-rachitic subjects; only in severe rickets (graded clinically and histologically) were reduced values found.[22,23] Unless the rachitic lesions be severe, therefore, chemical analysis of bone appears to be of little diagnostic value.

In view of the above difficulties, it would simplify the position if all four criteria for rickets could be used on the same patients. But since histological and chemical studies can be undertaken only on post-mortem material, and since it is very rare among Western peoples for young children to die with severe clinical rickets, such an investigation could be carried out only with the greatest difficulty.

(2) *Limitations of Studies on Children*

The only reliable method of determining the relationship between the occurrence and severity of rickets, and the habitual level of calcium intake, would be to induce rickets in young children on diets low only in calcium, and then to observe the effect on the lesions of appropriate additions of calcium salts. To deliberately induce rickets in children is, of course, out of the question. As Mackay[24] succinctly observed at a symposium on diseases of infancy held in London, "How many members would offer their babies as experimental animals for a study of the infants' requirements of this or that nutrient?"

In regard to therapeutic investigation, if deficiency of the element *is* implicated in rickets, then at least some ameliorating effect should be demonstrable with calcium supplements only. But in order satisfactorily to demonstrate and assess this therapeutic effect, several difficulties will be met. First, as already indicated, the position relating to the assessment of the severity of rickets is unsatisfactory. Uncertainties would therefore be present not only in choosing a comparable group of patients to be studied, but in assessing the progress made; in the British Pediatric Survey[18] referred to, there were frequent differences of opinion over the presence of "active" and "healed" rickets. Next, it would be essential to know the calcium status of patients. But as Darby,[25] also Goldsmith,[3] have noted, there is no present method of determining calcium status, i.e. calcium stores, and past and present calcium intakes. Finally, regarding treatment, difficulties involved in keeping, not only the diet, but the degree of exposure to radiation carefully controlled for protracted periods, will be appreciated. Unless all variables are kept to a minimum, clear-cut conclusions cannot be drawn.

The difficulties described thus show some of the obstacles which must be overcome before the rôle of calcium intake in rickets can be evaluated.

Quite apart from the uncertainties outlined, inherent in such an investigation, no study appears to have been made in which

the response to calcium has been compared with the response to vitamin D, or calcium plus vitamin D. Such a study was not included in the classical investigation of rickets in Vienna in 1919–1922,[26] possibly because the usual hospital diet, which was shown to have no healing effect on children with rickets, was already rich in calcium. That severe rickets can occur in the presence of ample calcium has, of course, been observed from early times.[6,12,26–30] The fact that the remedial effect of calcium has not been demonstrated satisfactorily does not, however, prove that a supplement of calcium has *no* effect on rickets. But in view of the large amount of interest taken in the etiology of rickets by able authorities of the past, Cheadle,[27] Findley,[28] Mellanby,[6] Hess,[11] Park,[29] and others, it would seem reasonable to consider that whatever healing effect is engendered by the addition of calcium only can be of little clinical significance.

Calcium Intake and Rickets in Populations and Population Groups

It is widely held that the requirement of vitamin D is influenced, *inter alia*, by the amount of calcium ingested; i.e., a high calcium intake requires less vitamin D to facilitate its absorption compared with a low intake, and vice versa.[31,32] In other words, some vitamin D can spare some calcium. However, as Jeans[33] has stressed, with decreasing calcium intake, vitamin D cannot compensate indefinitely, for a point must be reached where, even if all the calcium ingested were absorbed, the amount absorbed would be insufficient for satisfactory bone formation. But the critical level may well be lower than is generally imagined, for equilibrium and good retentions are known to occur at low levels of intake.[34–39] This is attained, first, by absorbing a greater percentage of calcium, and second, by reducing the amount excreted; i.e., retentions can be comparable to those obtaining with higher levels of intake.

What happens, then, to the incidence of rickets when the calcium intake is lowered, as often occurs in wartime, or, when it is habitually low? These two states will be considered separately.

The Incidence of Rickets among Populations whose Calcium Intake has been reduced

It must be constantly kept in mind that rickets is a disease of *growing* bone, for among children whose growth is retarded or arrested, rickets is not observed.[4,6,26–29] Now all diets which support growth *must* contain a modicum of calcium. If calcium intake be decreased, what point is reached first? Is it the point where insufficiency of the element causes bone to be poorly mineralized, or, is it the point where the concomitant lack of other nutrients (calories, proteins, etc.) causes a slowing down of growth, thereby automatically arresting the development of rickets? Were the former alternative valid, rickets would be a characteristic feature of undernutrition. But this is not so. Reviewing wartime experience as a whole, Keys *et al.*[40] have concluded that there was no increase in the incidence of rickets. Furthermore, under conditions where all nutrients were often severely restricted, as at Belsen,[40,41] Warsaw,[42] starving Holland in 1945,[43] and in the Japanese prisoner of war camps,[44] rickets was not observed. Evidently the diets consumed either had sufficient calcium available for satisfactory bone growth, or, before the level of calcium became too low, lack of other nutrients arrested bone formation and rickets did not supervene. It is apparent, therefore, that the belief that progressive lowering of calcium intake causes or promotes the development of rickets is not supported by wartime experience.

The Incidence of Rickets among Populations whose Calcium Intake is habitually low

Populations may be divided into two groups: (1) those dwelling in the tropics and semitropics, where the intake of calcium is habitually low, but where available radiation is plentiful; and (2) those dwelling in northerly countries where the calcium intake is higher, but where available radiation is less plentiful.

(1) Populations where Calcium Intake is low but Radiation plentiful.

In several regions in the tropics and semi-tropics, for example, East Africa,[45] Rhodesia,[46] Indonesia,[47] Burma,[48] and rural India,[49,50] although the habitual calcium intake is almost invariably low, clinical rickets is believed to be rare. In South Africa, in both rural and urban areas, such rickets is rare among the school child[51–53] and the younger population.[54] However, in crowded centers of population, in South Africa,[55–57] as well as in India,[49–50] rickets is common and occasionally severe. In Johannesburg, for example, rickets is frequently observed, but the majority of the patients are breast-fed Bantu babies whose intake of calcium is normal. After two years, when the child begins to move around and fend for itself, rickets is rarely seen, although at that time the child's calcium intake (in proportion to its accepted requirement) is at its lowest throughout the life period.[56,57] Gillman and Gillman[58] have noted their astonishment that gross bone deformity is not more widely prevalent in older Bantu children.

Concerning adult rickets or osteomalacia, no case of this disease in a pregnant Bantu woman has yet been reported from Central or Southern Africa. The disease is common in India,[50] though apparently only among women who practice purdah (seclusion). It is of interest to note that there is evidence that the poorest of Chinese women who are forced throughout the season to work in the fields do not get osteomalacia, despite the consumption of a most unsatisfactory diet characterized by its low calcium content.[59–61] Among Chinese women on a slightly higher social plane, and who can afford to remain indoors, particularly throughout the inclement winter, osteomalacia is common. There appears to be no evidence, therefore, that a low calcium intake causes or promotes the development of osteomalacia.

(2) Population Groups among whom Calcium Intake is higher, but available Radiation is less plentiful.

Unfortunately, no investigation on the incidence of rickets occurring exclusively among low consumers of calcium has been carried out. There are, however, a number of studies which suggest indirectly that the level of calcium intake is of little relevance in the disease. A few examples will be given. Glasgow is situated in latitude 58°; moreover, being an industrial city, reduction of ultraviolet radiation by smoke and smog must occur to an appreciable extent. In this city, one of the most comprehensive studies on rickets was carried out by Findlay and Ferguson in 1919.[62] A salient finding was that although the diet consumed by the non-rachitic children was only slightly superior to that of the rachitic subjects, of the latter, 40 per cent were not taken out regularly, while only 4 per cent of the non-rachitic children had been confined indoors regularly. More recently, Rustung[63] carried out a study of rickets incidence still further north in an inland Norwegian valley. The incidence of the disease was high, particularly up to school age (30–40 per cent). The diet was composed mainly of cereals and vegetables, with a variable though usually a small amount of milk. Rustung found that freedom from rickets was closely associated with the length of time the children spent in the open air.

Furthermore, the frequently observed and sometimes dramatic effect of season on the incidence of rickets, despite probable constancy of calcium intake, is pertinent. For example, in a study in wartime Denmark, Fredericia[64] reported that the incidence of rickets in the first year of life was 22 per cent in the spring, but only 1.4 per cent in the summer and autumn.

The examples given, while not demonstrating calcium intake to be of no importance, at least suggest that level of calcium intake has little etiological significance.

Briefly, then, the evidence on the population groups discussed here, although it is admittedly inadequate, provide no support for the belief that the level of calcium intake is an etiological factor in the development of rickets.

Comment

It will be apparent from what has been discussed that a low calcium intake *per se* does not *cause* rickets. It is possible that low consumers of calcium are more susceptible to vitamin D deficiency than are high consumers of the element: i.e., for an equal vitamin D deficiency, a subject habituated to a low calcium intake may develop rickets earlier and more severely than a subject used to a higher intake. But there appears to be no evidence in favor of this possibility. Certainly, among the reasons advocated for a high level of calcium intake (and no dietary standards body has yet adduced detailed clinical and pathological evidence in support of this), it would be unwarrantable to include prophylaxis against a deficiency disease whose relationship to calcium deficiency is so far from being established. It would be equally unwarrantable, as the writer has shown elsewhere, to recommend a high calcium intake to guard against the inhibiting effect of phytate phosphorus on the absorption of calcium,[65] to ensure adequate mineralization of bone,[22] or to safeguard against retardation of growth or stuntedness at maturity.[66]

In the light of the foregoing, it is submitted that the time is opportune for a re-examination of the whole position relating to calcium requirement and deficiency stigmata.

Summary

It is widely believed that a low intake of calcium can cause or predispose to the development of rickets.

To determine accurately the responsibility borne by a low level of calcium intake in the etiology of rickets is almost impossible, due to (1) the unfeasibility of carrying out a carefully controlled study, (2) uncertainties in the diagnosis of the disease, and (3) lack of a reliable method of assessing the calcium status of patients.

If calcium deficiency be implicated, calcium salts should evoke some ameliorating response. Such an effect has not been satisfactorily demonstrated.

Wartime experience has indicated that when calcium intake is reduced, even drastically, no increase in the incidence of rickets occurs. In tropical and semitropical countries where a low calcium intake is almost invariable, rickets is uncommon when advantage is taken of the available radiation. In countries less favored in the latter respect, no studies on rickets appear to implicate, even indirectly, the level of calcium intake as an influencing factor.

While there seems to be no evidence that a low calcium intake promotes, still less causes, rickets, the possibility that level of intake has a modicum of etiological significance cannot be completely excluded. It would seem wholly unwarrantable, however, to list prophylaxis against rickets as a reason for insisting on the high intakes of calcium at present recommended.

REFERENCES

1. Hegsted, D. M., Moscosco, I., and Collazos, C. C.: Study of minimum calcium requirements of adult men. *J. Nutrition* 46: 181, 1952.
2. Hegsted, D. M., Toulson, M. F., and Stare, F. J.: Role of wheat and wheat products in human nutrition. *Physiol. Rev.* 34: 221, 1954.
3. Goldsmith, G. A.: Application to Human Nutrition, in *Biochemistry and Physiology of Nutrition,* edited by Bourne, G. H., and Kidder, G. W., Academic Press Inc., New York, 1953, vol. 2, p. 505.
4. Shohl, A. T.: *Mineral Metabolism,* American Chemical Society Monograph series, Reinhold Publ. Corp., New York, 1939.
5. McCance, R. A., and Widdowson, E. M.: Mineral metabolism of healthy adults on white and brown bread dietaries. *J. Physiol.* 101: 44, 1942.
6. Mellanby, E.: *Experimental Rickets,* Spec. Rep. Ser. Med. Res. Coun. Lond., No. 61, 1921, and No. 93, 1925.
7. Youmans, J. B., and White, Patton E.: *Nutritional Deficiencies: Diagnosis and Treatment,* J. B. Lippincott, Philadelphia, 1941.
8. Macy, I. G., and Williams, H. H.: *Hidden Hunger,* Catell Press, Lancaster, Pa., 1945.
9. Bicknell, F., and Prescott, F.: *The Vitamins in Medicine* (3rd ed.), Heinemann, London, 1953.
10. Kirkpatrick, H. F. W., and Robertson, J. D.: Calcium and Phosphorus Metabolism, in *Biochemistry and Physiology of Nutrition,* edited

by Bourne, G. H., and Kidder, G. W., Academic Press Inc., New York, 1953, vol. 2: p. 410.

11. Hess, A. F.: *Rickets including Osteomalacia and Tetany*, Lea and Febiger, Philadelphia, 1929.
12. Holt, L. M., and Howland, J.: *The Diseases of Infancy and Childhood* (9th ed.), D. Appleton and Co., New York, 1931.
13. Sheldon, W.: *Diseases of Infancy and Childhood.* (6th ed.), J. & A. Churchill Ltd., London, 1951.
14. Neff, F. C.: Rickets, in *Practice of Medicine*, edited by Tice, F., W. F. Prior Co., Hagerstown, Md., 1945, vol. 9, p. 145.
15. Nicholls, L.: *Tropical Nutrition and Dietetics* (3rd ed.), Baillière, Tindall, and Cox, London, 1951.
16. Park, A. E.: quoted from Sherman, H. C., and Lanford, C. *Essentials of Nutrition* (3rd ed.), Macmillan, New York, 1952.
17. Youmans, J. B.: Mineral deficiencies. *J.A.M.A.* 143: 1252, 1950.
18. British Pediatric Association: *The Incidence of Rickets in War Time.* Reports on Public Health and Medical Subjects. No. 92. Ministry of Health, London, 1944.
19. Follis, R., Jackson, D., Eliot, M. M., and Park, A. E.: Prevalence of rickets in children between 2 and 14 years of age. *Am. J. Dis. Child.* 66: 1, 1943.
20. Follis, R. H., Park, E. A., and Jackson, D.: The prevalence of rickets at autopsy during the first two years of age. *Bull. Johns Hopkins Hosp.* 91: 480, 1952.
21. Hawk, P. B., Oser, B. L., and Summerson, H. W.: *Practical Physiological Chemistry* (12th ed.), Blakiston, Philadelphia, 1949, p. 1145.
22. Walker, A. R. P., and Arvidsson, U. B.: Studies on human bone from South African Bantu subjects. Part I, The chemical composition of ribs from subjects habituated to a diet low in calcium. *Metabolism* 3: 385, 1954.
23. Higginson, J.: Studies on human bone from South African Bantu subjects. Part II, Histopathological studies on the ribs of South African Bantu children. *Metabolism* 3: 392, 1954.
24. Mackay, H. M. M.: Nutritional needs of infancy. *Proc. Nutrit. Soc.* 2: 69, 1944.
25. McLester, J. S., and Darby, W. J.: *Nutrition and Diet in Health and Disease* (6th ed.), W. B. Saunders Co., Philadelphia, 1952, p. 118.
26. Chick, H., Mackay, H. M. M., Hume, E. M., Dalyell, E. J., Smith, H. H., and Wimberger, H.: *Studies of Rickets in Vienna, 1919–1922.* Spec. Rep. Ser. Med. Res. Coun. Lond., No. 77, 1923.
27. Cheadle, W. B.: Rickets, in *A System of Medicine* by Allbutt, T. C. Macmillan, New York, 1902, vol. 3, p. 108.
28. Findlay, L.: Etiology of rickets. *Lancet* 1: 956, 1915.
29. Park, A. E.: Etiology of rickets. *Physiol. Rev.* 3: 106, 1923.
30. Virtanen, A. I.: Nutrition of the people in Finland with special reference to vitamin intake. *Nord. med. Tidskr.* 15: 250, 1938. (Abst. in *Nutrition Abstr. & Rev.* 8: 180, 1938.)
31. Morris, N.: Vitamin D deficiency. *Proc. Nutr. Soc.* 1: 148, 1944.
32. Harris, L. J.: The antivitamins. *Brit. J. Nutrition* 2: 385, 1949.
33. Jeans, P. C.: in McLester, J. S., and Darby, W. J.: *Nutrition and Diet in Health and Disease* (6th ed.) W. B. Saunders Co., Philadelphia, 1952, p. 224.
34. Nicholls, L., and Nimalasuriya, A.: Adaptation to low calcium intake in reference to calcium requirements of tropical population. *J. Nutrition* 18: 563, 1939.
35. Basu, K. P., Basak, M. N., and Sircar, B. C. R.: Studies in human metabolism, Part II. Calcium and phosphorus metabolism in Indians on rice and on wheat diets. *Indian J. Med. Res.* 27: 471, 1939.
36. Kraut, H., and Wecker, H.: Kalkbilanz und Kalkbedarf. *Biochem. Ztschr.* 315: 329, 1943.
37. Kraut, H., and Wecker, H.: Kalkbilanz und Kalkbedarf. *Biochem. Ztschr.* 318: 495, 1948.
38. Walker, A. R. P., Fox, F. W., and Irving, J. T.: Studies in human mineral metabolism: effect of bread rich in phytate phosphorus on metabolism of certain mineral salts, with special reference to calcium. *Biochem. J.* 42: 452, 1948.
39. Walker, A. R. P.: Calcium balance studies on Bantu adults consuming their every-day low calcium diet. Unpublished work.
40. Keys, A., Brožek, J., Henschel, A., Mickelsen, O., and Taylor, H. L.: *The Biology of Human Starvation*, University of Minnesota Press, Minneapolis, 1950.
41. Lipscomb, F. M.: Medical aspects of Belsen concentration camp. *Lancet* 2: 315, 1945.
42. Hottinger, A., Gsell, O., Uehlinger, E., Salzmann, C., and Labhart, A.: *Hungerkrankheit, Hungerödem, Hungertuberkulose*, Benno Schwalbe, Basel, 1948.
43. Smith, C. A.: Effects of maternal undernutrition upon new born infants in Holland (1944–1945). *J. Pediat.* 30: 229, 1947.
44. Smith, D. A., and Woodruff, M. F. A.: *Deficiency Diseases in Japanese Prison Camps.* Spec. Rep. Ser. Med. Res. Coun. Lond., No. 274, 1951.
45. Trowell, H. C.: Medical examination of 500 African railway workers. *East. Afr. Med. J.* 25: 236.
46. Gelfand, M.: *The Sick African* (2nd ed.), Stewart, Cape Town, 1948.

47. LUYKEN, R.: Personal communication.
48. AUTRET, M. M.: The nutrition problem in Indochina. *Bull. Soc. Sci. Hyg. Aliment.* 36: 294, 1948.
49. HUTCHISON, H. S.: Rickets in India. *Lancet* 1: 377, 1922.
50. WILSON, D. C., and WIDDOWSON, E. M.: *Comparative Nutritional Survey of Various Indian Communities,* Indian Med. Res. Mem., No. 34, 1942.
51. KARK, S. L., and LE RICHE, H.: Health study of South African Bantu School children. *S. Afr. Med. J.* 18: 100, 1944.
52. *Annual Medical and Sanitary Report for Swaziland Colony, 1952.* Public Health Department, Mbabane, Swaziland.
53. SQUIRES, B. T.: *The Feeding and Health of African School Children.* Cape Town Sch. Afr. Studies. New Ser. No. 20.
54. FALCKE, H. C., WALKER, A. R. P., STRYDOM, E. S. P., and FLETCHER, D. C.: The incidence of rickets in young Bantu children in urban, peri-urban and rural areas. Unpublished work.
55. GILLMAN, J., and GILLMAN, T.: The problem of supplementing the African diet in the light of current concepts of nutrition. *The Leech* 17: 39, 1946.
56. FELDMAN, N.: Infantile rickets: its occurrence in Non-Europeans in Johannesburg. *S. Afr. Med. J.* 24: 1053, 1950.
57. KAHN, E.: Personal communication.
58. GILLMAN, J., and GILLMAN, T.: *Perspectives in Human Malnutrition,* Grune and Stratton, New York, 1951.
59. MAXWELL, J. P.: Osteomalacia in China. *Chinese Med. J.* 37: 625, 1923.
60. MILES, L. M., and FENG, C. T.: Calcium and phosphorus metabolism in osteomalacia. *J. Exper. Med.* 41: 137, 1925.
61. SNAPPER, I.: Calcium and Phosphorus Malnutrition, in JOLLIFFE, N., TISDALL, F. F., and CANNON, P. R.: *Clinical Nutrition,* Paul B. Hoeber, New York, 1950, p. 251.
62. FINDLAY, L., and FERGUSON, M.: *A Study of Social and Economic Factors in the Causation of Rickets.* Spec. Rep. Ser. Med. Res. Coun. Lond. No. 20, 1918.
63. RUSTUNG, E.: Studies on rickets, I. Occurrence of rickets in a Norwegian inland valley, in relation to diet, housing, climate and altitude. *Acta Pediat.* 17, Suppl. 2, p. 93, 1935 (abstract in *Nutrition Abstr. & Rev.* 5: 776, 1936.)
64. FREDERICIA, L. S.: Nutritional investigations in Denmark during the War, 1939–1945. *Proc. Nut. Soc.* 5: 255, 1947.
65. WALKER, A. R. P.: Cereals, phytic acid, and calcification. *Lancet* 2: 244, 1951.
66. WALKER, A. R. P.: Does a low intake of calcium retard growth or conduce to stuntedness? AM. J. CLIN. NUTRITION 2: 265, 1954.

Bread Faddism

"Another fad involving shaken confidence in the value of a food concerns white bread. In early America and Great Britain, when the cleanliness of food was a constant problem, whiteness of bread was tantamount to quality. In fact, the public demand for white flour often led to adulteration with alum, which not only whitened the loaves but also increased their size. Today an opposite demand is frequently encountered. Some people believe that white bread, as commonly purchased now, is nutritionally poor. This, of course, is to overlook the added milk vitamins, and minerals—not to mention the proteins, other nutrients, and calories of the white bread itself."

—W. H. Sebrell, Jr. *Federation Proceedings* 13: 780, 1954.

Biochemical and Nutritional Effects of Lysine-Reinforced Diets

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GERTRUDE M. HYDE, M.D.,‡ AND LOUISE ORTO, B.A.§

ANIMAL experiments by Mitchell[1] have indicated that the lysine needs of weanling rats may be more critical than those of adult rats. The amino acid requirement data of Mitchell[2] and Rose[3] for young adults and those of Albanese[4] for young infants indicate that a similar circumstance may prevail in the human. Calculations from these data disclose that the ratio of lysine to tryptophane needs of young adults is 2.0–3.2, whereas that of the young infant is 6.0. These values suggest that within the limitations of these data the lysine growth needs of the infant are twice as great relative to the tryptophane requirements as the maintenance needs of the young healthy normal adult.

Since tryptophane is probably the limiting essential amino acid in most infant foods; and since the lysine-tryptophane (L/T) ratios of most foods employed for infant feeding, with the possible exception of meat products, are lower than the growth L/T, it was felt that the nutrition of infants, especially those with poor appetites, could be improved by lysine supplementation of the diet. A report of our observations in an extended study of these effects is presented here.

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EXPERIMENTAL

Diets and Methods

The infants studied were fed evaporated or fresh milk formulae with supplementary foods, in accord with prevailing pediatric practice in this area. The caloric intake was maintained at 100–120 calories per kilogram, or 45–55 calories per pound per day, with an approximate percentile caloric distribution of proteins, 15; fats, 35; and carbohydrate, 50. The caloric density of the formulae employed was roughly one calorie per cc.

The data of Williamson[5] were employed for estimating the lysine and protein intake from fresh or evaporated milk. The protein and lysine content of all supplementary foods fed in this study was known from chemical and microbiological analyses performed directly on representative samples of the products employed. Analyses of detailed daily diet records in terms of these data disclosed the protein intake, from all sources, to average 3.5 Gm. per Kg. per day.

The vitamin and mineral intake was maintained at, or better than, recommended dietary allowance levels[6] by the use of good commercially available concentrates.* In addition to the vitamins of known dietary need, these preparations also provided pyridoxine, pantothenic acid, folic acid, and vitamin B_{12} in adequate amounts.

The reported observations were made on 15 infants (age 1–27 months) for periods varying from 9 to 21 weeks. The lysine-supplemented diet periods were from 3 to 4 weeks' duration and were preceded and followed by control

* Kindly supplied to us by Dr. Edward R. Neary of the White Laboratories, Incorporated.

periods of equal time. In several instances, because of obvious clinical needs of the child, the supplemental periods were repeated or prolonged. The supplement was given as L-lysine, HCl* at the rate of about 100 mg. of lysine per Kg. body weight per day. DL-lysine was not employed because the D-form is not utilized by the human.[4] To insure complete consumption and maximum utilization, the calculated amount of the amino acid was added during preparation of the milk formulae.

It was further noted by these investigators that the plasma levels of lysine, isoleucine, and histidine (in that decreasing order) underwent the severest reduction in malnourished individuals. As a prelude to undertaking the detailed metabolic studies outlined above, chromatographic measurements of free blood lysine and total plasma protein levels of 15 infants (age 1–13 months) were performed biweekly for 2 to 3 months. These determinations disclosed that, as in adults, the blood

TABLE I

Amino Acid Requirements of Young Adults and Infants*

	Young adults				Infants, Albanese[4]	
	Mitchell[2]		Rose[3]			
Amino acid	Amount mg./Kg.	Maintenance pattern	Amount mg./Kg.	Maintenance pattern	Amount mg./Kg.	Growth pattern
Tryptophane	6	1.0	7	1.0	30	1.0
Lysine	12	2.0	23	3.2	180	6.0
Threonine	14	2.3	14	1.9	87	2.9
Methionine	7	1.1	31	4.3	85	2.8
Isoleucine	17	2.3	20	2.8	90	3.0

* Growth and maintenance pattern figures were obtained by assigning the value of unity to tryptophane.

Anthropometric and body weight measurements were made at the beginning and end of each diet period. Blood specimens were also collected at these intervals for the microdetermination of total plasma proteins, hemoglobin, and amino acid levels. Multiple 24-hour urine collections were made intermittently during the course of the individual studies for the estimation of nitrogen balance. The amino acid content of the blood and urine were determined by chromatography.[7]

Comparable and parallel measurements were done on 9 infants (age 2–18 months) who received no lysine supplement during the period of observation, which varied from 3 to 20 weeks.

Results

Everson and Fritschel[8] have shown that in adults decreased levels of the total and individual essential free amino acids of the plasma may serve as an earlier index of malnutrition than the measurement of blood albumin levels.

* A synthetic product containing 95% L-lysine, HCl and 5% D-lysine. HCl generously supplied to us by Dr. N. W. Flodin of the Du Pont Company.

lysine level is generally a better index of the nutritional state of infants than is the plasma protein level. In addition, it was found from these serial tests that the lysine level served as a good indicator of daily protein intake. This was especially true in infants with poor appetites arising from a variety of causes.

Subsequently, additions of lysine (50–100 mg./Kg./day) to the diet of some of the infants in this group (who were malnourished in terms of body weight and the lysine or total amino acid index) were found to improve appreciably the rate of weight gain with no increase in total protein intake. These preliminary observations seemed to give experimental support to the theoretical deductions derived from the lysine/tryptophane (L/T) ratio concept.

The factual bases of this concept are presented in Tables I and II. It will be noted from Table I that the tryptophane needs of infants on a body weight basis, like the total protein needs, are four to five times greater than those of young adults. However, with the exception of lysine, the pattern of needs of the different amino acids is quite the same

TABLE II

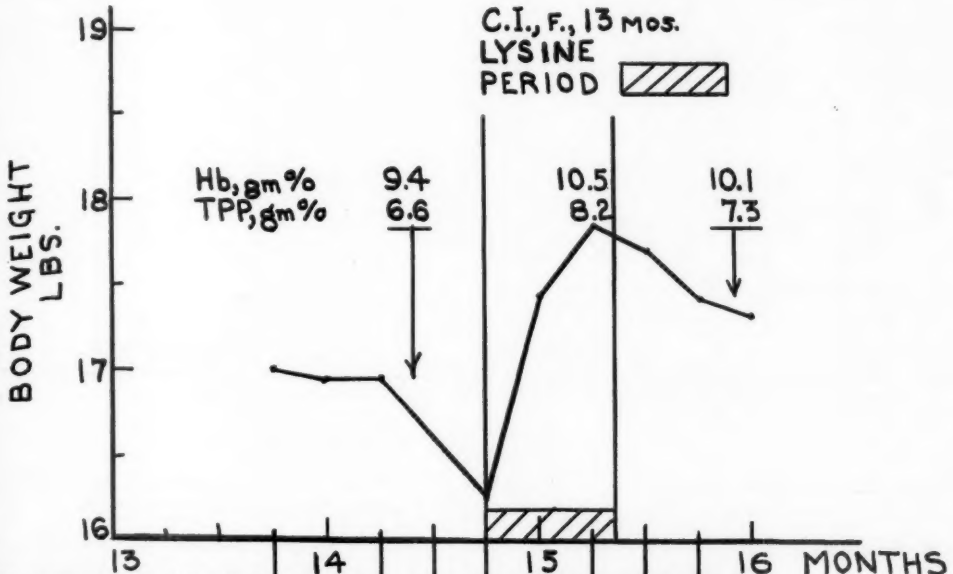
Comparison of Lysine—Tryptophane Ratios of Various Foods*

Infant food	Lysine/Tryptophane ratios
Human milk	3.4
Cow's milk	4.3
Soy products	1.0–3.4
Wheat products	2.5–2.7
Meat products	3.2–5.0
Cereal products	1.2–2.3
Protein digest preparations	3.0–3.9
Infant needs*	6.0

* Based on male infants 2–9 months of age, maintained on formula providing 3.5 Gm. protein and 100 calories per Kg. The formulae had a percentile caloric distribution: protein, 15; fat, 35; and carbohydrate, 50. The formulae provided all the known vitamins and minerals in adequate amounts.

for both infants and young adults. Examination of the lysine/tryptophane ratio content of foods commonly employed for infant feeding (Table II) discloses that only meat products approximate the infant L/T ratio.

In the light of these considerations, the results of the preliminary studies encouraged us to undertake a systematic evaluation of the nutritional effects of lysine supplements. Our study showed that of the 15 infants studied the body weight and nitrogen balance of 5 were markedly improved by the addition of lysine to the diet. The infants in this group ranged in age from 6 to 26 months and generally maintained poor appetites. In every instance, prior to determining the effect of lysine supplements, improved nutrition was attempted by increasing the protein content



DAILY PROTEIN INTAKE:	GM/LB.	2.4	2.1	1.4	1.4	2.2	2.3	2.3	2.3	2.2
	GM/KG.	5.4	4.5	3.1	3.2	4.8	5.1	5.2	5.0	4.9
LYSINE SUPPLEMENT:	GM/DAY	0	0	0	0	1.0	1.0	1.0	0	0
DIET L/T:		4.7	4.7	4.4	4.4	7.0	7.0	6.0	4.7	4.7

Fig. 1. Effect of lysine supplement on body weight and blood proteins of underweight child, who because of her aversion to solid foods was fed milk formula reinforced with milk protein preparations. High protein and high caloric diets were without effect except for reducing appetite of the child. Addition of lysine improved appetite and utilization of dietary protein.

of the diet (4 to 6 Gm./Kg.). In most infants the increased protein intake caused a transient increase in weight gain and nitrogen balance, which was soon followed by greater decreases in appetite and body weight. A good example of this phenomenon is shown in the data given in Figure 1. This infant was about 5 pounds underweight for her age. Attempts to improve her nutrition by high protein or high caloric diets prior to the period shown in the figure had met with consistent failure. However, when the lysine content of the diet was increased by supplementation, appetite was improved, high protein diets were better tolerated, and body weight increased. Concomitantly, the blood protein levels rose during the lysine-supplement period and fell in the post-control period. As might be expected from our previous experience, the free lysine levels of the blood were two to three times greater during the lysine period than in the pre- or post-control periods.

The data graphically collected in Figure 2 illustrate the application of lysine supplementation to a casein digest product (Nutramigen®). The use of this product was indicated by an allergic reaction in the infant to cow's milk in any form. Although the allergy symptoms (eczema) disappeared with the feeding of Nutramigen as 60–80% of the total protein intake, growth continued to be poor. The effect of the addition of lysine in changing the L/T ratio from 4.0 to 6.4 can be clearly seen in Figure 2. As before, the improvement with supplementation, and the decline in body weight after removal of lysine, were accompanied by parallel changes in blood protein levels. Attention is called to the lag effect which is sometimes observed both with the onset and termination of improved nutri-

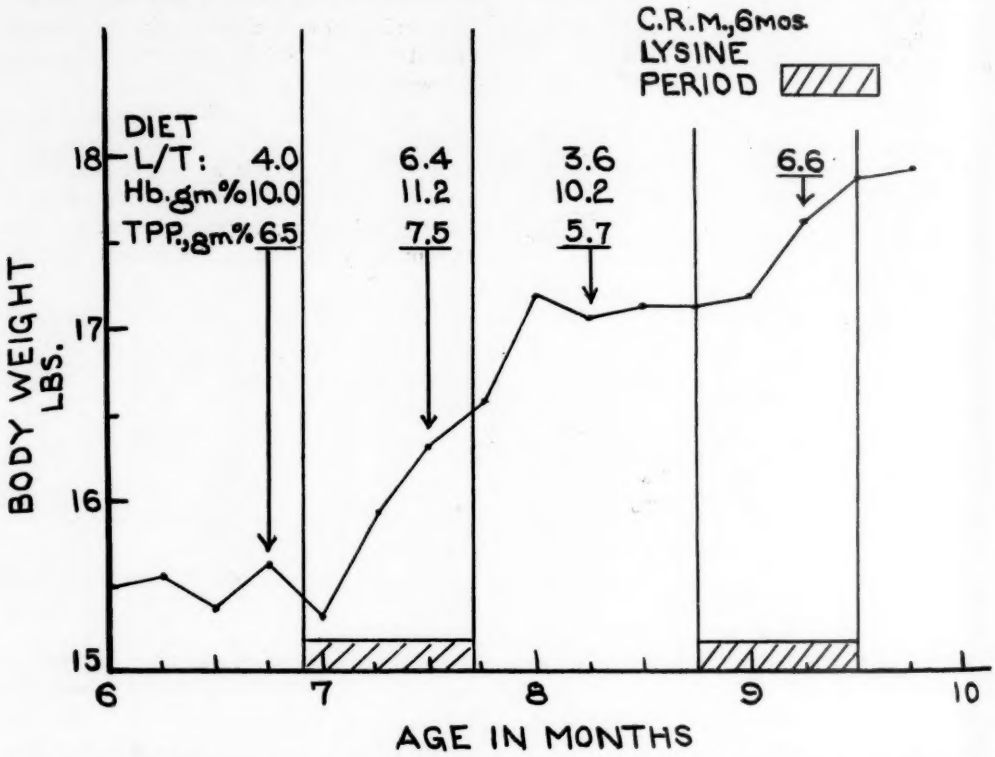


Fig. 2. Effect of lysine on nutrition of infant who because of allergic symptoms to cow's milk was maintained on a protein digest preparation (Nutramigen®) as a primary source of dietary nitrogen.

tion in relation to the periods of high lysine intake.

The prolonged effects of lysine supplementation are shown in an underweight child in Figure 3. Measurements based on multiple 24-hour urine collections at various intervals throughout the course of the study showed that improved body weight gain was paralleled by increased nitrogen retention. However, improvement was decreased by an intercurrent infection.

The other two infants of this first group showed similar marked responses to the addition of lysine to their diets.

The effects of lysine supplementation in the second group (6 infants, age 3–21 months) were characterized by significant and definite changes in the biochemical criteria, rather than by the marked body weight changes. A typical illustration of these findings is shown in Figure 4. It will be noted that a high nitrogen retention prevails, in spite of the limited body weight change. This seemingly anomalous observation is a rather common finding in infants.[9] It arises primarily from the possibility that tissue water and fat can be mobilized independently of body proteins.

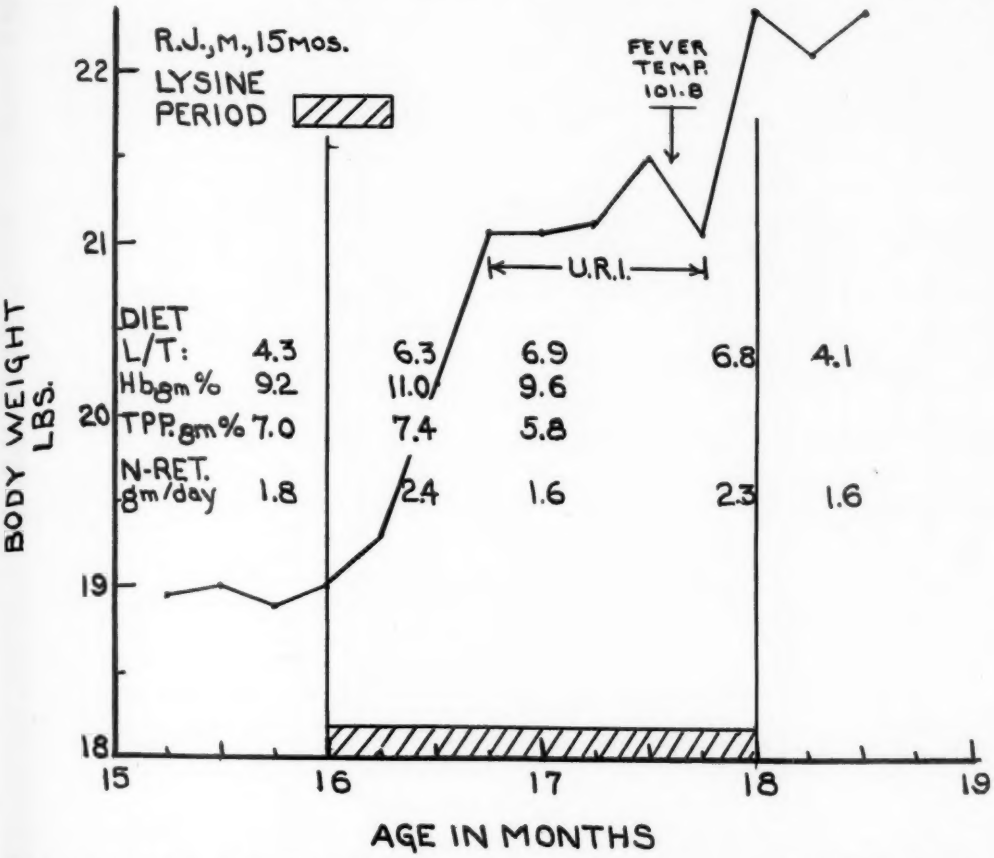


Fig. 3. Nutritional and biochemical effects of prolonged lysine supplementation on anorexic and underweight infant.

The significant biochemical changes associated in this group with increased dietary L/T ratios are shown in Table III. It will be seen therefrom that a rise in both hemoglobin and total plasma protein levels occurred with additions of lysine to the diet. Reductions in these values often occurred with cessation

of the supplement. The conclusions derived from the blood protein measurements were well supported by the findings on total amino acid and lysine levels of the blood.

The four remaining infants (age 2–6 months) of the 15 studied showed no improvement in any of the criteria employed as the result of lysine additions to their diet. This finding may be explained in part by the unusually high growth rate of these infants in terms of the Wetzel grid[10] or the University of Iowa standards.[11] They were also superior in this respect to our own control group of 9 infants.

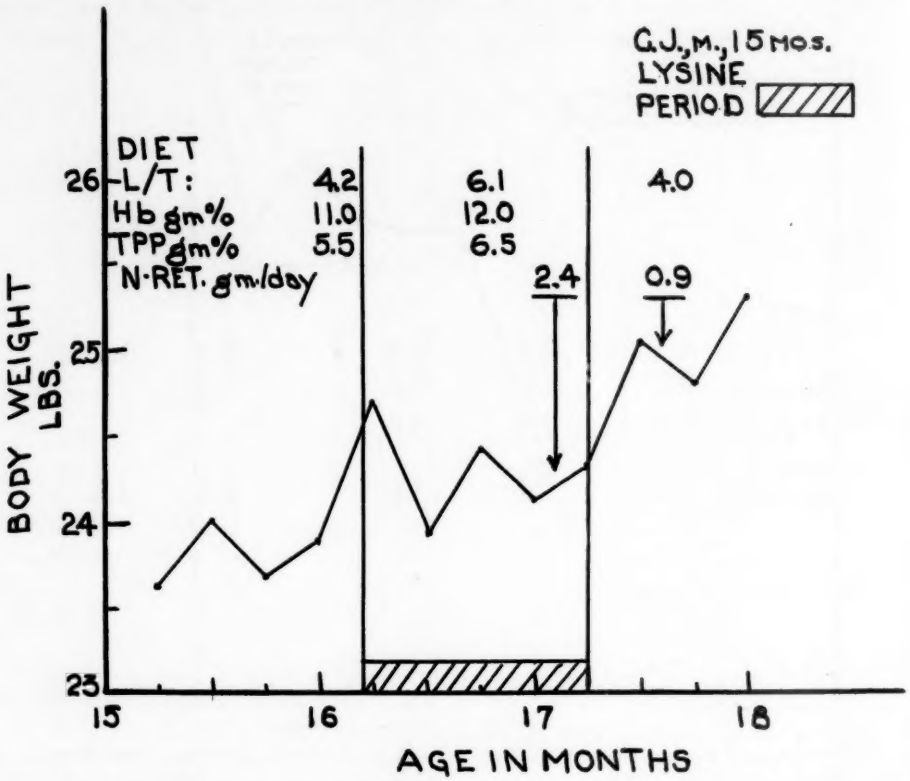


Fig. 4. Effect of lysine supplement on nitrogen retention of infant of average body weight and appetite. Dietary protein was derived in equal amounts from evaporated milk formulae and supplementary baby foods, e.g. meats, vegetables, etc.

Comments

The possibility that lysine supplementation of cereal foods, especially wheat products, would enhance their food value has long been recognized and amply demonstrated by animal and human experiments.[2] The reported findings now suggest that the nutritional value of many infant foods, including cow's milk, can be improved by small additions of lysine. This seemingly revolutionary idea arises from our newer knowledge of the specific amino acid needs of infants.

The nutritional limitations of feeding only milk and sugar beyond the sixth month of life (or earlier) have long been recognized by pediatricians in their recommendation for supplementary foods. Curiously enough, these recommendations include for the most part meat preparations which have high L/T ratios (Table II). These measures have been shown to succeed with infants of good appetite and to fail with infants of poor appetite. Recent evidence points to the fact that these failures

TABLE III

Effect of Lysine Supplements on Blood Protein Levels of Infants

Diet periods	Pre-control				Lysine Supplement				Post-control			
Subject, sex, age & initial body weight	Wks.	Diet, L/T*	Hb.† Gm., %	TPP‡ Gm., %	Wks.	Diet L/T	Hb. Gm., %	TPP Gm., %	Wks.	Diet L/T	Hb. Gm., %	TPP Gm., %
W. W., m., 3 mos., 10.9 lb.	3	4.8	10.0	4.8	3	8.0	11.0	7.3	4	4.7	10.4	6.8
B. V., f., 4.5 mos., 9.7 lb.	2	4.7	10.4	6.8	3	7.0	11.2	8.3	3	4.6	11.7	6.1
D. W., m. 5.0 mos., 10.5 lb.	4	4.3	9.1	6.6	4	6.5	10.0	7.1	5	4.0	8.3	6.4
C. G., m., 8.0 mos., 13.5 lb.	2	3.8	10.9	5.9	4	6.0	10.2	7.4	4	4.0	10.6	7.1
C. J., m., 15 mos., 23.5 lb.	3	4.1	11.5	5.5	4	6.3	12.1	5.6				
V. M., f., 24 mos., 17.5 lb.	4	4.2	9.2	7.4	8	6.3	11.5	7.2	3	4.1	11.0	6.3

* Lysine-Tryptophane ratio. † Hb.: hemoglobin. ‡ TPP: total plasma proteins.

may be associated with digestive enzyme deficiencies which prevail in young infants.[12] In the face of such circumstances, other methods for enhancing the nutritional value of milk need to be explored. The addition of easily assimilated lysine to infant formulae seems to offer one solution to the problem.

Summary

Studies were initiated to determine whether or not the nutritive value of milk could be improved by lysine supplements. Fifteen infants (1–27 months) were studied for periods ranging from 9 to 21 weeks. These children were fed standard fresh or evaporated milk formulae with supplementary foods in accord with prevailing pediatric practice. The vitamin and mineral content of the diet was as high as, or higher than, the recommended levels. The lysine-supplemented diet periods were from 3–4 weeks' duration and were preceded and followed by control periods of equal duration. The supplement was given as L-lysine, HCl at the rate of 100 mg. of lysine/Kg./day. The biochemical effects of the lysine supplements were determined in terms of anthropometric and body weight changes, nitrogen balance, hemoglobin, total plasma protein, and blood lysine levels. Comparable and parallel measurements were done on 9 infants (2–18 months) who received no lysine supplement during observation periods which ranged from 3–20 weeks.

The results of our studies showed that the body weight and nitrogen balances of 5 of the 15 infants were markedly improved by the lysine supplement. Six of the children showed no spectacular weight gains, but urinalyses showed that more nitrogen was retained than before the lysine was added, indicating that these children gained in strength and sturdiness. Blood protein levels also increased during this period. The remaining 4 infants showed no observable improvement when lysine was added to their diet, indicating that lysine-fortified diets are only effective with children who are not getting adequate nourishment from their food. The growth rate of these 4 infants was already well above average. The results of these studies bear the implication that the nutritional value of many infant foods, including cow's milk, can be substantially improved by small additions of lysine.

REFERENCES

1. Mitchell, H. H.: Some species and age differences in amino acid requirements, in *Protein and Amino Acid Requirements of Mammals*, edited by A. A. Albanese, New York, 1950, p. 9.
2. Bricker, M., Mitchell, H. H., and Kinsman, G. M.: Protein requirements of adult human subjects in terms of the protein contained in individual foods and food combinations. *J. Nutrition* 30: 269, 1945.

3. Rose, W. C.: Amino acid requirements of man. *Fed. Proc.* 8: 546, 1949.
4. Albanese, A. A.: Protein and amino acid requirements of man, in *Protein and Amino Acid Requirements of Mammals*, edited by A. A. Albanese, New York, 1950, p. 127.
5. Williamson, M. B.: Amino acid composition of human milk protein. *J. Biol. Chem.* 156: 47, 1944.
6. Food and Nutrition Board: *Recommended Dietary Allowances Revised 1953.* National Research Council, Publ. No. 302. Washington, D. C.
7. Albanese, A. A., Higgons, R. A., Vestal, B., and Stephanson, L.: Photochromatographic analysis of amino acids in body fluids. *J. Lab. & Clin. Med.* 37: 885, 1951.
8. Everson, T. C., and Fritschel, M. J.: The individual essential amino acids in plasma and urine of surgical patients. *Surgery* 30: 931, 1951.
9. Albanese, A. A., Holt, L. E., Jr., Irby, V., Snyderman, S., and Lein, M.: Studies on the protein metabolism of the infant. I. The comparative biological value of some milk protein preparations. *Bull. Johns Hopkins Hospital* 80: 149, 1947.
10. Wetzel, N. C.: The baby grid. *J. Pediat.* 29: 439, 1946.
11. Jackson, R. L., and Kelly, H. G.: Growth charts for use in pediatric practice. *J. Pediat.* 27: 215, 1945.
12. Douglass, F. H.: Why solid foods for newborn. *Northwest. Med.* 52: 832, 1953.

Food Propaganda and Food Consumption

"It is certainly most interesting that where the consumer has been at last given back his choice he has elected to spend his money on the highest quality protein that he can buy. It was not Hitler's bombs or his shadowy parachutists, or what have you, that daunted us during the early years of the war, but the frightful lumps of indigestible material which we were compelled to accept by way of sustenance at the hands of authority. No doubt authority had no option in the matter; rationing was unavoidable, and once rationing began choice naturally disappeared. All the same, it is an interesting commentary on the value—or lack of value—of propaganda. How many so-called experts were enlisted to persuade us that before the war we had eaten 'far too much meat,' that 'fish was better' for us, and that there was no nutritional or other discoverable difference between canned products and fresh? Of course, there has been a considerable easing off of recent years of this sort of nonsense, but even so, one might have expected that the lean years would have left a more prominent mark on our food habits. . . .

"Getting back to propaganda, it is quite clear that, however well intentioned, it is only successful if it is based on truth. The good habits, and they were many, that the Ministry of Food tried to inculcate during the war, have persisted, for again, according to the *Financial Times*, the consumption of milk, despite price increases, is still some 80 per cent higher than it was before the war. On the other hand, the consumption of bread has declined, though of the bread that is eaten the 'white loaf' of 70 per cent extraction flour, or lower, forms only 3 per cent of the total, and the 'national' loaf is supreme. That sterling but dull component of our menus—the potato—is also falling back toward its true level and is now very nearly at its pre-war consumption figure. . . . On the whole, then, the war has taught us to be a good deal wiser in what we eat, which is, one supposes, the only benefit we have derived from it."

—*The Medical Press* 232: 369-370, 1954.

On the Intravenous Administration of Cyanocobalamin

By SAMUEL GOLDBLATT, M.D., M.S.*

THERE IS general acceptance of the therapeutic effectiveness of intramuscular injections of crystalline cyanocobalamin (vitamin B_{12}) in a number of diverse pathologic states. The complete absence of side reactions (toxic or allergic) following this mode of therapy is very striking. The peroral administration of this drug, however, has caused a controversy based upon conflicting evidence as to its complete or effective absorption from the gastrointestinal tract.

The intravenous administration of cyanocobalamin has rarely been utilized, although it has, in my experience, advantages over the intramuscular injection, in certain circumstances. If the safety of intravenous administration can be shown to be as complete as that established for the intramuscular route, a technique of therapy becomes available to the clinician in which the rate and amount of absorption of the vitamin need not be taken into consideration.

Folkers[1] administered vitamin B_{12} intravenously to mice in doses as high as 1600 mg./Kg. without death or toxic manifestations. Such doses correspond to 112 million times the generally accepted daily human requirement of 1 μg. of cyanocobalamin. Ogasawara, Ata, and Hisada[2] reported that mice sustained the intravenous administration of cyanocobalamin in 15 μg. doses and rabbits in from 5 to 30 μg. amounts without toxic effect.

The first investigator who administered vitamin B_{12} intravenously to the human subject appears to have been Spies.[3] He gave cyanocobalamin intravenously to a group of patients with lateral sclerosis resulting from pernicious anemia, and concluded that the intravenous administration of this substance was both effective and non-toxic.[3a] His report was followed by that of Klima and Wengraf,[4] who gave daily intravenous doses of 15 μg. of vitamin B_{12} to 34 patients with pernicious anemia. They reported no hypersensitivity or toxic reactions. Chow[5] gave vitamin B_{12} intravenously in doses varying from 50 to 1000 μg. while studying the urinary excretion of this agent. No untoward results were noted. Similarly, Conley, Green, Hartmann, and Krevens[6] utilized 1000 μg. doses without undesirable side effect. Meyer, McInerny, and Ritz[7] also gave this material by vein in doses varying from 1 μg. daily to 7 μg. weekly, with satisfactory clinical response and without untoward reactions. In the treatment of three patients with pernicious anemia, Jännes[8] gave 75 μg. of vitamin B_{12} intravenously as single doses, without side effects. Goldeck and Weis[9] investigated the vitamin B_{12} activity in blood serum following the intravenous injection of 90 μg. of this material on nine successive days in three normal subjects, and in six with various diseases. All these individuals sustained the injections without side effect. In 1954, Morrison[10] utilized daily intravenous doses of 20 μg. of the vitamin as a lipotropic agent.

In 1950, I administered vitamin B_{12} intravenously in doses of from 15 to 30 μg. to determine the effect of this drug upon levels of vibratory perception in a group of normal subjects and in patients with various diseases. No side reactions were observed.

During an extensive investigation of the use of the intramuscular cyanocobalamin in the treatment of lupus erythematosus,[11,12,13] it appeared that perhaps even better results

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might be obtained by the use of this material intravenously in large daily doses.

Originally, crystalline cyanocobalamin (without preservative) in concentrations of 1000 μg. per ml.* was administered intravenously in daily doses of from 15 to 3000 μg. to a small group of patients. The complete lack of toxic or allergic reaction following the intravenous injection of this single-dose material suggested that the drug as found in multiple dose vials, containing 1.5% solution of benzyl alcohol as a bacteriostatic agent,† might be utilized. With this material also, no toxicity was noted, and, to date, 150 patients have been treated by the intravenous administration of 1000 μg. or more daily for varying periods of time, without toxic reaction. In some cases, the intravenous cyanocobalamin constituted only part of the treatment used.

The study includes 96 white and 7 colored females, and 43 white and 4 colored males, with an age range of from 4 to 78 years. Successive daily injections of 1000 μg. were given to a girl, age 10 years, for a period of over 270 days, without reaction. The largest single dose was 3000 μg., (white female, age 46, with uncontrollable pain after cervical laminectomy), given daily for a period of 2 weeks, interrupted for 1 week, and then repeated in similar courses for a total of 6 courses (86 intravenous injections of 3000 μg. each), without reaction.

Table I indicates the types of diseases treated, the variation in size of the individual dose, the number of doses given, and the total number of injections given to the patients in each group.

Case 47, B. H., white female, age 48, who was seen with an obscure condition previously mistakenly diagnosed as acute disseminated lupus erythematosus, described the occurrence of burning tongue, headache, and a post-nasal "drip" beginning ten minutes after injection of 1000 μg. of vitamin B_{12}, after having received numerous injections of this material previously without reaction. Observation of this patient for one hour immediately following the injection in which she described the burning of the tongue revealed no redness or swelling of the tongue or increased lachrymation, photophobia, or other signs of allergic reaction. Moreover, she has since then re-

TABLE I

Dosage of Intravenous Cyanocobalamin

Diagnosis	No. cases	No. of 1000 μg. inj.	No. of 2000 μg. inj.	No. of 3000 μg. inj.	Total no. inj.
Lupus erythematosus					
Acute disseminated	4	180	23		203
Subacute disseminated	3	95			95
Chronic disseminated	1	66			66
Chronic discoid	8	441			441
Photosensitivity	1	25			25
Psoriasis	11	222	4		226
Local neurodermatitis	10	111			111
Generalized neurodermatitis	2	31			31
Seborrheic dermatitis	5	116	6		122
Pyogenic infections	2	8			8
Viral infections	6	63	5		68
Fungus infections	5	94	5		99
Allergic manifestations	6	45	2		47
Contact dermatitis	13	140			140
Vesicular dermatoses	4	50	1		51
Acneform eruption	21	152			152
Alopecia areata	4	50	1		51
Vitiligo	3	51			51
Scleroderma	3	59			59
Endocrine disorders	18	278	3		281
Migraine	4	60	4		64
Severe neurologic diseases*	8	707	3	86	796
Malignancies	3	79			79
Miscellaneous†	5	31			31
Total	150	3154	57	86	3297

* Crystalline vitamin B_{12} in 1000 μg./ml. without preservative, supplied through the kindness of Dr. Nathaniel Ritter of Merck and Co., Rahway, N. J.

† Crystalline vitamin B_{12} made by Durr Products, Inc., Dayton, Ohio.

* In one case of juvenile syringomyelia the first 29 doses were of 500 μg. each.

† Miscellaneous cases include the following: pruritus ani (3); hyperhidrosis (1); pityriasis rosea (1).

ceived numerous injections of this vitamin without any complaints, so that it is probable that the administration of the drug was not responsible for the symptoms.

Aside from this very questionable reaction, none of the patients revealed subjective complaints or objective findings which might be construed to be allergenic or toxic effects from the 3297 intravenous injections of cyanocobalamin.

No attempt will be made here to evaluate the therapeutic effect of this mode of therapy as contrasted with that obtained by the administration of this agent orally or intramuscularly. The clinical results will be described elsewhere.

Summary

This report is presented to indicate that the intravenous administration of vitamin B_{12}, with or without bacteriostatic preservative (benzyl alcohol), is painless, non-toxic, and without allergenic side reactions. No reactions were noted in the course of 3297 intravenous injections among 150 patients with various diseases. The dosage ranged from 15 to 3000 μg. The clinician may, in my opinion, safely utilize this route of administration.

REFERENCES

1. Brink, N. G., Kuehl, F. A., Jr., and Folkers, K.: Vitamin B_{12}: the identification of vitamin B_{12} as a cyano-cobalt coordination complex. *Science* 112: 354, 1950.
2. Ogasawara, K., Ata, S., and Hisada, S.: Vitamin B_{12} and acetylcholine shock. *Lancet* 1: 598, 1953 (Letter to Editor).
3. Spies, T. D.: Recent progress in nutrition. *Postgrad. Med.* 6: 96, 1949.
3a. Spies, T. D.: Personal communication to the author.
4. Klima, R., and Wengraf, G.: Effect of vitamin B_{12} in 34 cases of pernicious anemia. *Wien. med. Wchnschr.* 101: 7, 1951 (abstr. *J.A.M.A.* 145: 1302, 1951).
5. Chow, B. F.: Sequelae to the administration of vitamin B_{12} to humans. *J. Nutrition* 43: 323, 1951.
6. Conley, C. L., Green, T. W., Hartmann, R. C., and Krevens, J. R.: Prolonged treatment of pernicious anemia with vitamin B_{12}. *Am. J. Med.* 13: 284, 1952.
7. Meyer, L. M., McInerny, R., and Ritz, N. D.: Intravenous treatment of pernicious anemia with vitamin B_{12}. *Science* 120: 75, 1954.
8. Jännes, J.: Observations on the excretion of vitamin B_{12} from the human system during the efforts tests, and preliminary studies on the vitamin B_{12} level of human blood plasma. *Acta med. Scandinav.*, suppl. 287: 65, 1953.
9. Goldeck, H., and Weis, C.: Vitamin B_{12} activity in blood serum after intravenous administration. *Klin. Wschr.* 31: 860, 1953.
10. Morrison, L. M.: Treatment of cirrhosis of the liver—recent innovations. *Am. J. Gastroenterol.* 21: 27, 1954.
11. Goldblatt, S.: Treatment of lupus erythematosus with vitamin B_{12}. *J. Invest. Dermat.* 17: 303, 1951.
12. Goldblatt, S.: Cyancobalamin (vitamin B_{12}) therapy of lupus erythematosus; further observations. *Acta dermat.-venereol.* 33: 216, 1953.
13. Goldblatt, S.: Studies in pallesthesia. Depression of vibratory sense levels in lupus erythematosus. *J. Invest. Dermat.* 22: 97, 1954.

Hyperchylomicronemia and Hypercholesterolemia: Their Correlation with Clinical Atherosclerosis

By THADDEUS D. LABECKI, M.D.*

IT APPEARS to be futile to expect that eventually one will be able to attribute the cause of human atherosclerosis to a single factor which from then on would be associated with atherogenesis. The entity which we term atherosclerosis differs only in degree—though often profoundly—from a condition which is present throughout our lives, evidence of which may be found in discrete, isolated intimal lesions in children and even in infants. Only the accelerated, advanced, or unfortunately located lesions are labeled clinically as "atherosclerosis." Whatever the intermediate morphologic and biochemical stages, the final outcome—the atheromatous plaque—is the result of a number of factors, each of which makes a definitive but unequal contribution. More and more evidence is accumulating to indicate that defective lipid metabolism is the determining factor as far as the degree of the disease is concerned. Should one or more of the other contributing factors co-exist, the result is early and/or advanced intimal atherosclerosis.

The traditional approach in both human and animal experimentation has been to estimate certain lipid fractions, using more or less reliable biochemical techniques. The study on experimental animals offers the advantage of visual demonstration—after sacrificing—of the presence, absence, and degree of lesions. Study of the physical makeup of the blood lipids has been neglected until relatively recently. Moreton's[1] contribution pointing out the postprandial increase of chylomicrons, Zinn and Griffith's[2] observations pertaining to the size of the particles, and the introduction of ultracentrifugation,[3] permitting the division of the lipoproteins into several classes according to the flotation rate, are notable and valuable exceptions to the traditional chemical approach. The classic experiments of Hueper,[4,5] in which he injected into the circulation foreign substances such as pectin, gum acacia, polyvinyl alcohol, and methyl cellulose, and produced intimal infiltration by those substances, give considerable food for thought on the manner in which the lipoprotein particles find a way into and become retained by the intima of the arterial tree.

The lipids in circulating blood are present in suspension in the form of lipoprotein macromolecules; the entire spectrum of the lipids consists of molecules and aggregates of various sizes and densities. According to the technique employed, the lipid spectrum can be divided arbitrarily into several segments. Since the microscope offers the simplest tool in that respect, one could divide the lipid macromolecules into two groups—visible and invisible segments. In the visible group, one should place those lipid macromolecules and aggregates which can be demonstrated utilizing the dark field microscope as studied by Gage and Fish,[6] Frazer and associates,[7,8] Moreton,[9] Zinn and Griffith,[2] Necheles,[10] and Labecki.[11] The submicroscopic spectrum can be studied with the aid of an ultracentrifuge[12] and classified according to the flotation rate, which depends on size and density.

Frazer and associates[7] were the first to point out that brightness appears to be a particularly impressive characteristic in dividing the visible macromolecules into two categories: dull particles, all of which are small (some on the margin of visibility), and bright ones, which they termed *chylomicrons*. The size of the latter, as cited,[7] varies from 0.3 to 0.5 micron

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in diameter. The chemical structure of chylomicrons according to Jones and associates,[13] citing unpublished data by Lindgren and Nichols, is composed of about 5 per cent cholesterol, about 5 per cent phospholipids, and a similar amount of protein, with from 75 to 80 per cent of glycerol ester. The study of Necheles[14] demonstrated that the washed chylomicron fats showed the presence of 10 to 12 per cent sterols, of which about 80 per cent was proved to be cholesterol, 65 per cent of the cholesterol being in esterified form.

TABLE I

Composition of Groups Under Observation

Age group	Non-infarction group					Infarction group				
	Total	WM	WF	CM	CF	Total	WM	WF	CM	CF
Under 45 years	28	8	20	0	0	14	13	0	1	0
45–54 years	49	19	7	9	14	20	17	1	1	1
55–64 years	42	7	9	17	9	29	22	5	1	1
65–74 years	34	16	14	2	2	11	8	2	1	0
75 years and over	63	13	50	0	0	0	0	0	0	0
Not stated	0	0	0	0	0	3	2	0	1	0
Total	216	63	100	28	25	77	62	8	5	2

Gofman and associates[15] reported a correlation between clinically demonstrable atherosclerosis and increased lipoprotein concentration, not in the class containing the greatest amount of cholesterol (S_f 3–8), but in the S_f 12–20 class. Subsequent reports from that group implicated an even broader spectrum, including S_f 20–100.

The provoking and bold theories of Moreton, the experimental results of Hueper, the early work of Zinn and Griffith, and my own work have encouraged us to study a group of individuals in order to correlate their known vascular disorders, or presumed health, with certain changes in the lipid makeup.

Materials

The subjects were divided into two categories: the infarction and non-infarction groups (Table I). The infarction group consisted of 77 patients, all of whom presented unequivocal electrocardiographic evidence of myocardial infarction in the past. Some of them, in addition, suffered from angina pectoris, but no subject was included in the infarction group on the basis of angina pectoris or history alone and/or purely clinical evidence, however suggestive it might have been. There were 216 subjects in the non-infarction group; of these, 139 were 55 years of age or older. The individuals with the lower age limit set arbitrarily at 55 could be considered as "control" subjects in the sense that they have survived long enough to allow manifestations of marked or accelerated atherosclerosis to occur. The 77 subjects under the age of 55 were investigated to study the effect, if any, of age on the chylomicron concentration in fasting serum. Those under the age of, say, 45 represent individuals whose "atherosclerotic destiny" is as yet unknown.

Sixty-three control cases 75 years of age or older were also gathered, to enable us to study the lipid pattern of those who survived the vascular perils of the fifth and sixth decades. In the control group females predominated, 125, as against 91 males; in the infarction group, there were 67 males and only 10 females.

The greatest number of cases of infarction in this series occurred in the 55–64 years age group, with the second largest in the 45–54 group. There were also 14 cases under the age of 45 years. The smallest number of cases occurred in the 65–74 years age group, and there were no infarction cases in the group 75 years of age and over. For the purpose of this report, only fasting chylomicron levels and total and esterified cholesterol are considered.

Methods

The subject is instructed to have the last meal at lunchtime the day preceding the test and to eat nothing in the evening but tea with a little sugar but no cream and a piece or two of dry toast. On the morning of the test the subject is not allowed to drink water or smoke, since both can produce peristalsis and affect the absorption of any residue in the intestine.

Approximately 10 cc. of venous blood are collected. This amount is necessary to provide adequate serum for the entire "lipogram," consisting of nephelometry, determination of chylomicron concentration, certain blood chemical determinations—total and esterified cholesterol, and, in a number of cases, total protein studies. To include ultracentrifuge studies, 50 cc. of blood are collected. Immediately after the blood has coagulated, the tube is "rimmed" carefully and centrifuged for 15 minutes at 2000–2500 r.p.m.; the serum is transferred to a clean tube and recentrifuged for 10 minutes at the same speed.

When performing microscopic examination of serum, it is important that the slides and cover slips be chemically clean to eliminate all possible artifacts. This may be accomplished by soaking them in dichromate solution, rinsing repeatedly, then allowing them to stand in absolute methyl alcohol or 95 per cent ethyl alcohol until used. Just prior to use they should be wiped completely dry with a lint-free cloth. A capillary tube is used to transfer one small drop of serum to a clean, dry slide. The serum may be deposited by blowing gently. The cover slip must be pressed down firmly to insure removal of air bubbles and a consistent depth of material. After a thorough check to be sure that the microscope is clean and free of dust particles and grease, and that the dark field condenser is properly centered, the slide is placed on the stage, using a drop of immersion oil on the condenser and a drop on the cover slip. The microscope is first focused on a square distant from the counting square, the counting square being most conveniently the one just below and to the right of the center ruled area in the net micrometer; it should be noted that particles showing Brownian movement are clearly defined. Once the counting square is in true focus, the vision is transferred to the counting square. It will be noted that some of the lipid particles are larger and more brilliant than others which are definitely smaller, duller, and poorly visible—the latter characteristics only infrequently are due to the globule being in a deeper plane. The larger particles (chylomicrons) are of approximately $^1/_8$ micron in diameter and larger; the smaller particles cannot be measured accurately, but that consideration is of minor importance. The number of chylomicrons and the total number of visible particles per square are noted. Counting must be rapid, as Brownian movement causes particles to move from one square to another with considerable speed, and the adjustment must not be changed while counting a predetermined square. Ordinary double mechanical counters should be used. When this square has been counted, the stage is moved to bring another field into view and the counting procedure repeated until 200 squares have been counted. The concentration (expressed in per cent) is determined by dividing the number of chylomicrons noted by the total number of visible particles and multiplying the value by 100. If the count is begun in the upper left corner of the cover slip, the entire area can be systematically covered in performing the count.

As equipment for routine counts we use a Spencer analytical binocular microscope with dark field condenser and oil immersion objective 1.8 mm, numerical aperture 1.25, giving the initial magnification of 95×. Total magnification is about 2000 diameters with an appropriate eyepiece. In one of the oculars we insert a net micrometer made by American Optical Company, calibrated so as to have the distance between two fine lines of about one micron. A strong bulb in a regular microscope lamp provides adequate illumination. The new Leitz equipment with an arc light is useful for more detailed study of lipomicrons. The latest Leitz monocular with a $^1/_{16}$ fluorite oil objective A = 1.32; 114:1, and the 25× ocular gives a magnification of about 3000 diameters. For particle size determination, the distance between two fine lines of the net micrometer is determined for any given objective-eyepiece-tube length combination. A Neubauer hemocytometer or a similar counting chamber may be used for that purpose.

Total and esterified cholesterols were determined by a modified Bloor's technique, using one ml. of serum made up to 25 ml. with Bloor's reagent. The aliquots were dried and extracted and the color developed by the Lieberman-Burchard reaction as originally set forth, but with very careful attention to factors such as time and temperature. Leitz and Photovolt lumetron colorimeters were used for reading the determinations.

Results

Most of the individuals tested had tests performed at varying intervals so as to obtain the mean value for a given individual. From those levels the mean values for the entire group were computed. When the data summarized in Table II were subjected to statistical analysis, it was found that both total cholesterol and chylomicron concentration were significantly higher in the infarction group as compared with the non-infarction group (persons 55 years of age or older without demonstrable evidence of intimal atherosclerosis). The difference between the means for the total cholesterol of the two groups was

four times higher than the standard error of difference. That difference for fasting chylomicron levels was 6.7 times the standard error. The similar value for esterified cholesterol was 1.7 (P = 0.009). The number of individuals taken under consideration in each of these groups may be expected to be sufficient for statistical evaluation.

TABLE II

Mean Value, Standard Deviation, and Standard Error of Mean of Chylomicron Level and Total and Esterified Cholesterol

Descriptive measurements	Non-infarction Group*	Infarction group
Chylomicron level		
Persons tested	136	75
Mean, %	35.78	42.76
Standard deviation	7.55	7.11
Standard error of mean	0.65	0.82
Cholesterol		
Persons tested	137	68
Mean, mg. per 100 ml.	266.73	304.86
Standard deviation	56.72	67.92
Standard error of mean	4.85	8.23
Cholesterol esters		
Persons tested	128	66
Mean, mg. per 100 ml.	188.40	200.78
Standard deviation	43.23	50.71
Standard error of mean	3.82	6.24

* Excludes all persons under 55 years of age.

The influence of the age factor is presented in Tables III and IV. The differences between the means in various age groups among the infarction group patients are not statistically significant; nevertheless, the overall trend is of interest. The cholesterol levels were highest in the 45–54 years group, while the greatest number of infarctions occurred in the 55–64 years group, with the 45–54 group second.

In the non-infarction group the difference between the means for total cholesterol in persons 65 years and over and persons under 45 was three times the standard error (P = 0.003). There was borderline significance between the 65-and-over age group and the 45–54 age group (2.4 times the standard error,

TABLE III

Mean Value, Standard Deviation, and Standard Error of Mean of Chylomicron Level* in Various Age Groups

Age Group and Descriptive Measurements	Infarction Group	Non-infarction Group				
		Total	Males	Females	Whites	Non-Whites
Total number of persons	75**	211	90	121	158	53
Mean	42.76	33.86	29.95	36.76	33.85	33.88
Standard deviation	7.11	7.71	6.42	7.33	8.21	6.03
Standard error of mean	0.82	0.53	0.68	0.67	0.65	0.83
Persons under 45 years	14	27	7	20	27	0
Mean	43.89	28.92	28.03	29.24	28.92	—
Standard deviation	13.18	7.32	—	8.24	7.32	—
Standard error of mean	3.52	1.41	—	1.84	1.41	—
Persons 45–54 years	19	48	28	20	25	23
Mean	43.97	31.16	28.95	34.27	27.60	35.04
Standard deviation	13.36	7.71	9.68	10.23	9.12	9.92
Standard error of mean	3.06	1.11	1.83	2.29	1.82	2.07
Persons 55–64 years	28	42	24	18	16	26
Mean	41.55	31.57	30.90	32.46	30.30	32.35
Standard deviation	10.93	6.26	8.35	10.93	10.78	8.55
Standard error of mean	2.06	0.96	1.70	2.58	2.70	1.68
Persons 65 years and over	11	94	31	63	90	4
Mean	43.71	37.67	30.54	41.17	37.69	37.18
Standard deviation	15.09	7.26	6.22	4.80	7.38	—
Standard error of mean	4.54	0.75	1.12	0.60	0.78	—

No significance tests were made with groups which had less than 10 persons. When the two groups under 55 years were combined into one group the same results were obtained in all comparisons.

* Chylomicron levels are expressed in per cent of the total number of visible particles.

** Includes 3 persons of unknown age.

TABLE IV

Mean Value, Standard Deviation, and Standard Error of Mean of Total Cholesterol in Various Age Groups

Age Group and Descriptive Measurements	Infarction Group	Non-infarction Group				
		Total	Males	Females	Whites	Non-Whites
Total number of persons	68*	208	86	122	155	53
Mean	304.86	258.07	241.85	269.50	261.60	247.75
Standard deviation	67.92	55.20	42.98	59.83	55.72	52.30
Standard error of mean	8.23	3.83	4.64	5.42	4.48	7.18
Persons under 45 years	14	26	7	19	26	0
Mean	283.14	227.77	237.43	224.21	227.77	—
Standard deviation	96.57	67.18	—	70.91	67.18	—
Standard error of mean	25.82	13.17	—	16.26	13.17	—
Persons 45–54 years	18	45	24	21	22	23
Mean	331.83	249.20	245.46	253.48	251.54	246.96
Standard deviation	99.69	45.89	66.76	76.15	73.73	68.97
Standard error of mean	23.51	6.84	13.62	16.63	15.72	14.37
Persons 55–64 years	24	40	24	16	14	26
Mean	300.64	256.74	242.92	277.47	284.18	241.96
Standard deviation	97.19	54.17	67.74	95.35	95.41	69.91
Standard error of mean	19.83	8.57	13.82	23.84	25.51	13.71
Persons 65 years and over	10	97	31	66	93	4
Mean	303.27	270.86	239.24	285.70	270.03	290.00
Standard deviation	128.31	57.18	39.87	58.10	55.50	—
Standard error of mean	40.60	5.80	7.16	7.16	5.76	—

No significance tests were made with groups which had less than 10 persons.

* Includes 2 persons of unknown age.

P = 0.016). The peak in the non-infarction group is reached in the oldest group, while in the infarction group it is reached in the 45–54 age group. The highly significant difference between the overall mean values in males and females in the non-infarction group was apparently due to the much higher (4.6 times the standard error, P = 0.000004) cholesterol levels in females 65 years and over than in males in a comparable age bracket. While in non-infarction males there was no apparent influence of the age factor, females 65 and older had significantly higher levels than females under 45 years of age. The intermediate groups, while from the purely statistical standpoint exhibiting no significant difference, showed in females a steady increase with the progress in age.

When comparing the infarction cases with the non-infarction subjects, the total cholesterol levels were higher in all age groups in the infarction patients, with statistical significance (3.4 times the standard error, P = 0.007) in the 45–54 age group and borderline significance (2.0 times the standard error, P = 0.05) in the 55–64 age group.

The overall trends in the esterified fraction of cholesterol were similar to those of total cholesterol. The peak was reached in the infarction patients in the 45–54 age group; in the non-infarction patients in the 55–64 age group; total cholesterol continued to climb with age.

The decrease in the esterified cholesterol in the oldest non-infarction group was not statistically significant. Again, females 65 and over had significantly higher levels (2.5 times the standard error) than males in the same age group; similarly, females under 45 had lower ester levels than females in the 55–64 and 65-and-over age groups. In the non-infarction group, the influence of age was perhaps more significant in the esterified fraction of cholesterol than when total cholesterol was considered: individuals under 45 had lower levels than those in the 55–64 age group (difference between the means was 2.9 times the standard error) and similarly lower (2.5) than the 65-and-over age group. There was borderline significance between the under-45-years age group and the 45–54 age group (2.3 times the standard error).

The difference between the means in the infarction group versus the non-infarction group was less striking in the esterified fraction than in total cholesterol, the difference being 2.4 times the standard error when overall levels were considered and 2.1 times in the 45–54 age group.

Somewhat more spectacular differences existed when the ratio of chylomicrons (particles about 0.3 micron in diameter and larger) to the *total* number of visible fatty particles was considered. Comparing the overall figures, the difference between the means of the infarction and non-infarction groups was 9.2 times the standard error. When subjects were divided into groups according to age, the patients with infarction showed surprisingly similar mean ratios in all age groups, and the ratios were always statistically significantly higher than the mean group values for the non-infarction subjects. The exception was in the 65-and-over age group—43.7 as compared with 37.6, the mean of the non-infarction group in that age bracket.

In the remaining groups, the chylomicron levels were comparable in both sexes. While the differences were not statistically significant, it is interesting that the non-infarction males reached their chylomicron peak in the 55–64 age group; females exhibited a sudden and highly significant increase in the 65-and-over age group.

When one considers the range within which the chylomicron levels may be found, it is indeed striking that the mean values for all age groups among the infarction patients are very similar to each other, and as a group, much higher than in the non-infarction subjects. This is also seen when the total cholesterol levels of the infarction cases are analyzed.

Discussion

This study, and a number of others on the relation of probably abnormal lipid metabolism to human atherosclerosis, should be considered in the light of an apt statement by Patterson[16]: "to use coronary artery disease as a criterion in these studies means that one is measuring not only atherosclerosis but extraneous factors as well." The division of subjects into two categories, "myocardial infarction and non-infarction," contains an inherent error common to all human experiments in the field of atherosclerosis, because, unquestionably, a small group of atherosclerotic individuals had the misfortune of having atheromatous plaques in such a location and accompanied by such hemodynamic events that the infarction occurred without the coexistence of marked generalized atherosclerosis. On the other hand, the control group must include individuals who have developed over a period of years sufficient collateral circulation to prevent them from having *clinically demonstrable* atherosclerosis. Conservatism is therefore in order in the interpretation of lipid abnormalities. Similar attitudes should prevail among those who pursue animal experimentation; grossly artificial conditions are created in order to produce lipid infiltration, and there are often marked morphological differences between human and animal "atheromata."

It is accepted by most investigators that the source of lipid material deposited in the intima and sub-intima is the circulating blood. Whatever the reason for the deposition, these fatty substances enter initially in the physical and chemical state in which they circulate. Under ideally "normal" conditions, all of the substances not needed for immediate use by the vascular wall tissue either re-enter the stream or are perhaps partly drained by the channels passing through media and adventitia. It would be up to the morphological school to give us a conclusive picture of local processes in health and in disease. We can speculate that a fine interplay of many factors exists whereby, among other substances, fatty particles, more or less loosely bound to protein molecules, enter the intercellular space, aided by varying degrees of permeability of the intima and by lateral pressure of the column of blood. Should that fine balance which normally allows, and perhaps indeed compels, these substances to be removed be disturbed, some of the lipoprotein molecules or aggregates would become enmeshed. What these factors are we can again only speculate. We know that hypertension aids the development of atherosclerosis. A greater influx of lipoproteins or a retarded reflux, or both, can thus be caused by increased intravascular pressure.

Some animal experimentations suggest that the absolute levels of certain lipids may be increased and still the atherosclerosis-like processes retarded. Thus the physical state of those lipids—colloidal dispersion, electrical

charge, sheer size, or perhaps even the shape of the molecules—may play a considerable role.

Furthermore, the degree of permeability of the intima, as regulated by hormonal balance, has to be considered. Be that as it may, some of the fat becomes enmeshed, and stimulates tissue activity comparable to a foreign body response.

The relationship of various lipid fractions in the atherosclerotic lesions (particularly early lesions) approximates that normally found in the serum.[17] While cholesterol and its esters may comprise even less than half of the material contained in early lesions, it eventually gains the upper hand, occupying about 70 per cent of late lesions, the rest being neutral fat, phospholipids, and protein fractions. This and other observations are responsible for the time-honored association between cholesterol and atherosclerosis.

It must be remembered that cholesterol is distributed over the entire spectrum of lipid molecules which, unlike other substances in serum, exist in the state of colloidal suspension. The greatest content of cholesterol is found in the smallest molecules exhibiting lowest flotation rate and highest density. Their level in serum was reported by Gofman not to correlate with the incidence of myocardial infarction. That correlation was reported to exist between the molecules having somewhat smaller cholesterol content, greater size, higher flotation rate (S_f 12–20 and S_f 20–100) and lower density. Progressing to even greater size along the lipoprotein-macromolecule spectrum, we enter the microscopic segment visible under the described conditions in the dark field. Their flotation units are in the magnitude of thousands; their size varies from somewhat less than 0.1 micron (limit of visibility with incidental light and optimal wave length) to well over 1 micron; their cholesterol content is relatively small, probably not over 12 per cent (Necheles), their content being mainly neutral fat with an unknown proportion of phospholipids and proteins. They could be compared to macromolecular substances like polyvinyl alcohol, pectin, gum acacia, and methyl cellulose, which Hueper injected into animals, producing intimal infiltration.

Whatever other local and systemic factors are responsible for the lipid (or more correctly, lipoprotein) infiltration of the intima, a deviation from the optimal range of relative concentration of those various segments in the lipoprotein-molecular spectrum is likely to be reflected in the atherogenetic processes. Various methods designed to detect those deviations are not competitive in any sense; on the contrary, they are complementary and not infrequently demonstrate the same phenomenon analyzed by two or more avenues of approach.

The suspected error in lipid metabolism may very well manifest itself in one or more deviations from normality. Such an abnormality may be assumed from the purely chemical approach, for example, when total cholesterol is found to be strikingly elevated. The abnormality may be in the increase in the "ultracentrifugal" macromolecules which contain a considerable amount of cholesterol and its esters. But, on the other hand, there may be only an increase in fatty particles 0.3 micron in diameter and larger, as we found to be the case in a number of subjects. Although the latter contain smaller amounts of cholesterol, that fact may be offset by the absolute rise of this fraction which can also be reflected in the results obtained by the purely chemical approach. It is equally probable than an individual may have some deviation from the ideally normal relationship of those fractions, but that other factors may favor more efficient removal of the lipoprotein macromolecules, or that their entry is relatively retarded by decreased tissue permeability. Then the defense mechanism may be sufficient to protect the entry of the greater than normal number of "heavies" or, should they enter, they may be disposed of in a more efficient manner.

An interesting similarity may be noted between our data and that of Gertler and Oppenheimer.[18] Their paper, and ours,[11] were read on the same day before the Gerontological Society. Their mean total cholesterol levels in women and men past 65 years of age were 282 and 226 mg. per 100 ml., respectively (Schoenheimer-Sperry method); our results were 285 and 239 mg. per 100 ml. (modified Bloor's technique). The esterified fraction in Gertler's

study shows mean values of 208 mg. for women and 167 mg. per 100 ml. for men, while ours were 193 and 164 mg. per 100 ml. The chemical approach of Gertler and Oppenheimer also revealed higher mean values of neutral fat in females as compared with males (227 and 183 mg. per 100 ml.), although they do not claim statistical significance for that fraction. As was pointed out previously, our study revealed significantly higher chylomicron levels in females 65 years of age and over as compared with males in the same age group. Since chylomicrons contain a considerable amount of triglycerides, it is not surprising that our biophysical approach correlates with the biochemical study of Gertler and Oppenheimer.

Perhaps it requires re-emphasis that females 65 years of age and over without *clinically* demonstrable coronary artery involvement were the only "non-atherosclerotic" subjects who showed hyperchylomicronemia; in the group comparison between the individuals with clinically demonstrable atherosclerosis and the non-atherosclerotic subjects that group of elderly females was included. In spite of the fact that these women do show high chylomicron levels, the low levels in the younger age groups and in non-atherosclerotic males in all age groups caused the difference between the atherosclerotic and non-atherosclerotic subjects to be statistically highly significant.

Summary

The application of dark field microscopy to the study of lipids in fasting human serum is discussed. The results obtained in 77 cases of myocardial infarction and 216 non-infarction cases are presented and statistically analyzed for degree of correlation with the incidence of myocardial infarction. Statistically higher chylomicron levels were found in the myocardial infarction cases when compared with non-infarction individuals of the same age group and sex, except in the 65 years-and-older group. Peak levels of chylomicrons and total cholesterol were observed in the 45–54-year-olds in the infarction group, while in the non-farction group this peak was found in the oldest age groups. Women in the age group 65 years and over, with no demonstrable clinical evidence of atherosclerosis, were found to have significantly elevated chylomicron levels when compared with younger men and women. The levels of total cholesterol were found to show significant correlation with the incidence of myocardial infarction in this series of cases.

Acknowledgment

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REFERENCES

1. Moreton, J. R.: Atherosclerosis and alimentary hyperlipemia. *Science* 106: 190, 1947.
2. Zinn, W. H., and Griffith, G. C.: Study of serum fat globules in atherosclerotic and non-atherosclerotic male subjects. *Am. J. M. Sc.* 220: 597, 1950.
3. Pederson, K. O.: *Ultracentrifugal Studies on Serum and Serum Fractions.* Almqvist and Wiksells Boktryckeri, Upsala, 1945.
4. Hueper, W. C.: Organic lesions produced by polyvinyl alcohol in rats and rabbits: toxicopathologic investigation of experimental thesaurosis. *Arch. Path.* 28: 510, 1939.
5. Hueper, W. C.: Arteriosclerosis. *Arch. Path.* 39: 117, 1945.
6. Gage, S. H., and Fish, P. A.: Fat digestion, absorption, and assimilation in man and animals as determined by dark-field microscope, and fat soluble dye. *Am. J. Anat.* 34: 1, 1924.
7. Frazer, A. C., and Stewart, H. C.: Ultramicroscopic particles in normal human blood. *J. Physiol.* 90: 18, 1937.
8. Elkes, J. J., Frazer, A. C., and Stewart, H. C.: Composition of particles seen in normal human blood under dark-ground illumination. *J. Physiol.* 95: 68, 1939.
9. Moreton, J. R.: Physical state of lipids and foreign substances producing atherosclerosis. *Science* 107: 371, 1947.
10. Marder, L., Becker, G. H., Maizel, B., and Necheles, H.: Fat absorption and chylomicronemia. *Gastroenterology* 20: 43, 1952.
11. Labecki, T. D.: A study of serum lipids in atherosclerotic and apparently healthy subjects. *J. Gerontol.* 7: 484, 1952.
12. Gofman, J. W., et al: Role of lipids and lipoproteins in atherosclerosis. *Science* 111: 166, 1950.
13. Jones, H. B., et al: Lipoproteins in atherosclerosis. *Am. J. Med.* 11: 358, 1951.

14. NECHELES, H.: Peptic ulcer in man: new antacid made to meet requirements of antacid therapy, chemical and laboratory work. *Am. J. Digest. Dis.* 18: 1, 1951.

15. GOFMAN, J. W., ET AL: Blood lipids and human atherosclerosis. *Circulation* 2: 161, 1950.

16. PATTERSON, J. C.: Factors in production of coronary artery disease. *Circulation* 6: 732, 1952.

17. WEINHOUSE, S., and HIRSCH, E. P.: Chemistry of atherosclerosis: lipid and calcium content of intima and of media of aorta with and without atherosclerosis. *Arch. Path.* 29: 31, 1940.

18. GERTLER, M. M., and OPPENHEIMER, B. S.: The serum lipid pattern in women and men from 65 years of age. *J. Gerontol.* 7: 484, 1952.

What Happens to Our Food?

"In any investigation science exerts its prerogative to break down the total complex event into simple parts. The scientist focuses his attentions on limited sequences, and partial aspects. If he tried to preserve the unity entire, he would remain hopelessly bogged down. For example, if we ask, what happens to the food we eat?, we are asking a question too complex for an intelligible answer. To make any reply meaningful, we must analyze the problem into simpler parts. We must specify what happens *where*. In the mouth? In the stomach? In the small intestines? Or in the liver, the pancreas, or the blood stream? We must specify *what* food. Fats? Carbohydrates? Proteins? And in what quantity? Starvation diets, average amounts, or surfeits? And then of course, *when?* At what points in the time-scale? At what age, at what temporal relation to exercise, or stress? All of these questions reflect our fundamental interest, of trying to isolate the relevant conditions that attend the phenomenon. But there are so many relevant aspects; what is relevant for one person might not be significant for another. If everyone had the same sort of liver or the same sort of pancreas, investigation would be a lot simpler. But they don't. Some individuals, for example, have stones in the gall bladders. Others have very small amounts of thyroid gland secretion. Still others harbor amoebae in their intestinal tract. In reply to our question, what happens to the food we eat?, our answer will depend, in part, on whether the individual has stones in the gall bladder or amoebae in his colon."

—Lester S. King. *Philosophy of Science* 21: 193, 1954.

Effect of a Therapeutic Regime on Hyperchylomicronemia and Hypercholesterolemia

By THADDEUS D. LABECKI, M.D.,* I. B. BRIGHT, M.D., W. W. LAKE, M.D., AND C. C. THOMPSON, M.D.

THE PRIMARY object of this investigation was to study some of the effects of the so-called lipotropic substances on fat metabolism in general and on disease conditions allegedly associated with derangement of fat metabolism.

There are two possible approaches to the study of serum lipids. The lipoid substances are distributed over the wide spectrum of the colloidal suspension; these lipoprotein macromolecules range from the most minute ones first fractionized by Pederson[1] to the largest chylomicrons well within the visible spectrum first studied in greater detail by Frazer and associates.[2,3] Thus, through the extraction methods, one may ascertain the total amount of a given lipid material contained in various proportions in the macromolecules of varying size and density; or, using as a yardstick some of the physical properties, one may attempt to determine the level of a given class of suspended lipoprotein molecules. In this study, both approaches have been applied, using the size of the particles and the overall levels of total and esterified cholesterol as indices.

A distinction has been made in this report between atherosclerotic patients (cases of proved myocardial infarction) and presumably "normal" subjects. This clinically arbitrary division was dictated by two facts: (1) elevated serum cholesterol levels have been reported to correlate with an increased tendency toward atherosclerosis[4,5]; (2) the increased tendency to myocardial infarction appears to correlate in a statistically significant manner with elevated fasting chylomicron levels.[6-9]

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MATERIALS AND METHODS

Sixteen patients with electrocardiographically proved myocardial infarction in the past were initially put on a lipotropic regime. All but two of them were males and all but one Caucasian. Their mean age was 48.8 years. Of this group, 11 remained on the lipotropic regime for nine weeks or longer, and the data on these 11 are considered for evaluation.

Because we have observed a significant increase of the chylomicron levels in presumably non-atherosclerotic females 65 years of age and older, it was deemed advisable to administer the substances to 26 white females with a mean age of 77 years, all of whom are permanent residents of the Old Ladies Home. Of this group, 25 remained on the regime for four weeks, 25 for nine weeks, and 12 for twelve weeks or longer. Up to the time of this report, five control subjects, non-psychotic in-patients at a State Hospital, have been receiving a placebo for four to six weeks. We expect to follow a larger group for a longer period.

Determinations of total serum cholesterol, esterified cholesterol, and chylomicron concentration form the basis of this report. The cholesterol determinations were made according to modified Bloor's technique. Chylomicron concentration refers to the percentage of lipoprotein particles, 0.3 micron in diameter and larger, microscopically visible under proper dark field setup. Our technique was a modification[7] of the technique cited by Zinn and Griffith[6] and was uniformly carried out by the same personnel.

At least two laboratory workups were performed on all the subjects prior to administra-

tion of lipotropes. All tests were performed on fasting (18 to 20 hours) serum. If any marked deviation occurred, the sample was discarded and the test repeated. No changes of diet, daily habits, etc., were instituted. On our advice several subjects interrupted administration and resumed it again, repeating this procedure several times. Any such change was immediately preceded by a laboratory study.

As this represents the first phase of our experiment, it was considered advisable to administer mixed lipotropic material*, as this approach appeared to have some experimental justification, particularly in the realm of hepatic therapy[10–13]. The arbitrary average daily dose (6–8 capsules per day) provided about 2 Gm. of choline dihydrogen citrate, 800 mg. of methionine, and over 600 mg. of inositol, together with vitamin B_{12} and liver extract. (A study is also being conducted on the effect of individual lipotropic substances administered alone.) Only the subjects who followed the regime strictly were considered.

Results

The problem was studied and the results interpreted by appropriate statistical methods. Changes were evaluated carefully, considering both the normal variability of serum cholesterol levels and chylomicron concentrations in fasting serum and the range of error in the techniques employed. Intermittent periods of therapy served as controls in the same individual.

Usually two independent groups of measurements are compared for significance by using their means and standard deviations to determine whether the ratio of difference between the means to the standard error of the difference of the means is larger or smaller than 2.5 (P = 1 per cent). Since the two groups of values in these tests are not independent, but are on the same individuals tested at separate times, a different method of analysis was used. The method takes into account individual variations rather than group variations, in that the difference between the values before the initiation of the regime and the levels of each person after administration is compared with the mean difference of the "before" and "after" levels for the group. Their standard deviation measurement ("S") was computed by dividing the sum of the squares of the deviations of each individual difference from the overall mean difference by the number of persons measured, less one, and then extracting the square root of the number:

$$S = \sqrt{\frac{\text{sum of squares of deviations}}{n - 1}}$$

Significance was determined by using the mean difference, the "S" value, and Snedecor's "Z" chart,* which estimates the number of pairs of observations necessary for significance. Tables I and II list the values obtained and their respective significance.

In both the infarction and control groups there was a statistically significant depression of chylomicron levels (Table I). This effect seems to become demonstrable three to four weeks after initiation of the regime and continues during the period of administration and for a short time following discontinuation. This "after effect" varies from individual to individual but usually lasts for several weeks. It should be pointed out that the depression appears to be greater in the infarction group than in the non-infarction group as the regime continues. This may be significant, inasmuch as the initial mean chylomicron level for the non-infarction group was 44.56, and for the infarction group 45.15. The similar values of total cholesterol were 316.02 and 307.41, and for cholesterol esters 211.42 and 217.75—all mg. per 100 ml.

In the myocardial infarction group, prolonged administration of the drug (nine weeks and longer) resulted in borderline statistically significant lowering of total serum cholesterol levels (Table II). There was little or no ap-

* Methischol, supplied through the courtesy of U. S. Vitamin Corporation.

* The detailed description of this statistical technique may be found on page 37 of *Statistical Methods* by George W. Snedecor, published in 1940 by Ohio State College Press.

TABLE I

Effect of Administration of Lipotropic Substances on Fasting Chylomicron Levels

Period of administration, weeks	4	9	12
Non-infarction group			
Number of persons	25	24	11
Mean difference	−5.56	−10.36	−8.19
"S" value	4.73	6.34	3.22
Statistical significance	Yes	Yes	Yes
Period of administration, weeks	**Up to 9**		**9 or More**
Infarction group			
Number of persons	11		11
Mean difference	−7.94		−15.72
"S" value	6.16		7.57
Statistical significance	Yes		Yes

− signifies decrease.

TABLE II

Effect of Administration of Lipotropic Substances on Total Serum Cholesterol and Cholesterol Esters

Period of administration, weeks	4	9	12
Non-infarction group			
Cholesterol			
Number of persons	25	24	12
Mean difference	+11.16	−0.02	+0.29
"S" value	20.80	20.42	26.50
Statistical significance	Borderline	No	No
Cholesterol esters			
Number of persons	25	24	12
Mean difference	−6.22	+0.79	+4.38
"S" value	20.16	25.27	43.23
Statistical significance	No	No	No
Period of administration, weeks	**Up to 9**		**9 or More**
Infarction group			
Cholesterol			
Number of persons	12		11
Mean difference	−13.46		−25.44
"S" value	30.42		37.50
Statistical significance	No		Borderline
Cholesterol esters			
Number of persons	12		11
Mean difference	+2.25		−14.80
"S" value	28.37		46.50
Statistical significance	No		No

− signifies decrease; + signifies increase.

parent effect on the cholesterol levels when the regime was continued for a period of less than nine weeks. During the first four weeks of administration of the lipotropic compound to the non-atherosclerotic group, there was an increase in total cholesterol levels of borderline statistical significance; subsequent periods of the regime were characterized by minimal variations in the cholesterol levels.

As was mentioned before, the non-infarction subjects were purposely chosen from among females over 65 years of age, since we found that these individuals exhibit chylomicron levels comparable to the values of atherosclerotic subjects in much younger age groups. Thus the starting point in the two groups under consideration in this study was strikingly similar.

The differences obtained, if considered due to the regime instituted, should be viewed against the known mean variability in the chylomicron concentration. This mean for 31 individuals tested on repeated occasions during the first year of our long-range project was 2.3 per cent; for 16 individuals repeatedly tested by the same technical personnel during the second year of the study, it was 1.7 per cent, giving the overall mean of 2.0 per cent. The split samples of the *same test* performed blindly rarely differ by more than 2.0. In our hands, a comparable mean value for cholesterol variation was 17.9 mg. per 100 ml., and 12.9 mg. per 100 ml. for the esterified fraction of cholesterol.

The third test of significance was carried out on four subjects from among the infarction group. The results are presented in Figure 1. It is apparent that the degree to which the lowering of pre-regime chylomicron levels can be obtained varies considerably from subject to subject, but the trend is similar in all of them. This fact is particularly brought out by intermittent discontinuation of the regime.

The figures in Table III show the results in five patients currently under placebo administration.

Discussion

The visible lipoprotein macromolecules and aggregates are only part of a long spectrum of which chylomicrons are the "high end." The

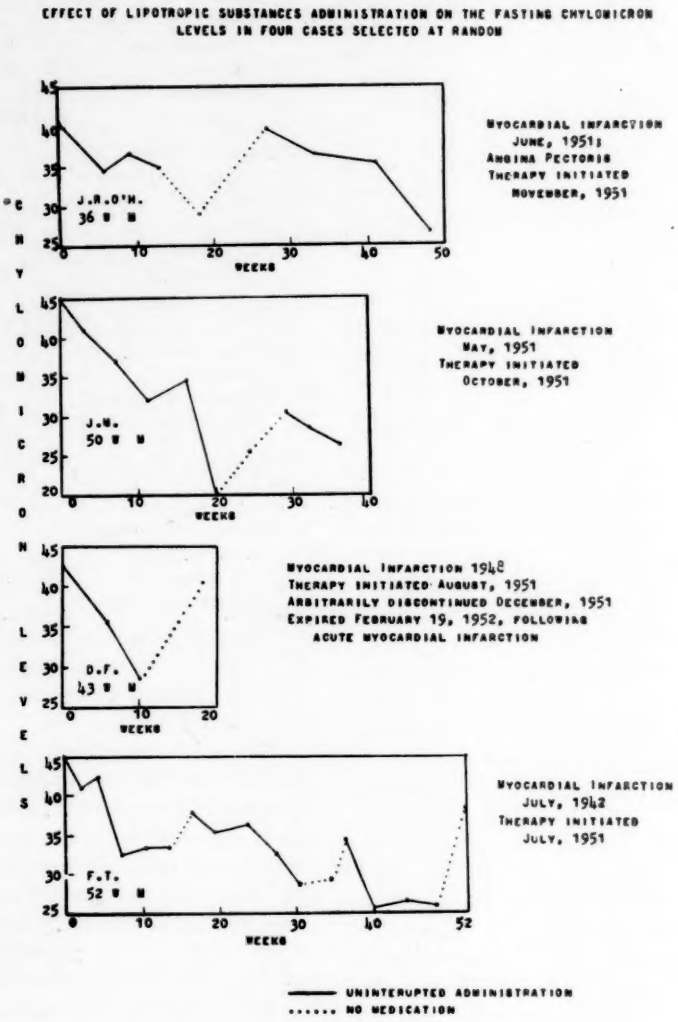
EFFECT OF LIPOTROPIC SUBSTANCES ADMINISTRATION ON THE FASTING CHYLOMICRON LEVELS IN FOUR CASES SELECTED AT RANDOM
CHYLOMICRON LEVELS
WEEKS
J.R.O'H. 36 W M
MYOCARDIAL INFARCTION JUNE, 1951; ANGINA PECTORIS THERAPY INITIATED NOVEMBER, 1951
J.W. 50 W M
MYOCARDIAL INFARCTION MAY, 1951 THERAPY INITIATED OCTOBER, 1951
D.F. 43 W M
MYOCARDIAL INFARCTION 1948 THERAPY INITIATED AUGUST, 1951 ARBITRARILY DISCONTINUED DECEMBER, 1951 EXPIRED FEBRUARY 19, 1952, FOLLOWING ACUTE MYOCARDIAL INFARCTION
F.T. 52 W M
MYOCARDIAL INFARCTION JULY, 1942 THERAPY INITIATED JULY, 1951
UNINTERRUPTED ADMINISTRATION
NO MEDICATION

Figure 1

TABLE III
Results of Placebo Administration to Five Patients

Patient	Initial chylomicron level (average)	Length of administration	Level after administration
M. L. W.	40.2	4 weeks	43.8
J. A. G.	46.2	6 weeks	44.8
E. L. S.	29.7	7 weeks	34.6
R. C. McB.	39.2	9 weeks	40.4
R. M.	32.4	1 week	33.3
		5 weeks	33.6

reproducibility of results on the fasting serum (as contrasted with *varying* chylomicron levels in the post-prandial period) reflects the base line which an individual exhibits. The presence of fatty substances is explained by the fact that the systemic lipids do not comprise an inert mass but are part and parcel of a dynamic system. It appears from the classic experiments of Schoenheimer and associates that before fatty acids of dietary origin can be oxidized they are first incorporated into the body fat depots; there is a simultaneous removal of fatty acids from those depots, and this change is both constant and dynamic. This was demonstrated by tagging fatty acids with deuterium. Fats are transported in the form of myriads of macromolecules; the most minute ones studied with the aid of an ultracentrifuge (they may be termed ultramicroscopic) contain considerable amounts of cholesterol per macromolecule, in addition to small amounts of tri-glycerides and proteins (globulin fraction or fractions) and moderate amounts of phospholipids. The greater the size of the particle, the smaller the content of cholesterol, phospholipid, and proteins, with an increase in the neutral fat fraction. A statistically and experimentally significant change in the level of a given fraction may represent a shift caused by the increase or decrease of the total amount of substances such as cholesterol and phospholipids. The reflection of that purely chemical change, often difficult to demonstrate by present chemoanalytical methods, is found in the change of respective levels of various fractions created arbitrarily by dividing the lipoprotein molecular spectrum into segments within the "ultramicroscopic" part (studied ultracentrifugally) and the "microscopic" one (studied in the dark field). The lowered phospholipid-cholesterol ratio of Ahrens and Kunkel might suggest either too much cholesterol (considerable amounts of which are contained in lipoprotein macromolecules on the low end of the spectrum) or too little phospholipids. If the latter be the case, let us remember that a higher level of chylomicrons reflects large amounts of triglycerides per particle, with much smaller concentration of phospholipid. That again would tally with the observation of Zinn and Griffith[6] and Labecki[7,8,9] on the correlation between elevated chylomicron levels and the incidence of myocardial infarction.

It would be premature at present to imply that lowering of chylomicron levels (percentage of particles 0.3 micron in diameter and larger, in relation to all *visible* lipoprotein macromolecules) has a preventive or curative effect. However, our past and current studies indicate the constancy of the chylomicron level under normal circumstances (no special dietary or drug regime) and a very good statistical correlation between elevated chylomicron levels and the occurrence of myocardial infarction. The effect of lipotropic agents in lowering the chylomicron levels appears to be surprisingly consistent. Whether or not the action of choline and methyl group donors (methionine, betaine) and inositol is to provide components of phospholipids and thus increase the phospholipid serum levels can only be speculated. Such an increase might be reflected from the physical standpoint by the increase in the smaller (0.1 micron to 0.2 micron) visible fatty particles—and perhaps others in the ultramicroscopic segment—thus causing a relative decrease in the chylomicron concentration. The action of certain substances may create a shift in the levels of lipoprotein macromolecules of various sizes. This might be the explanation of the suggested cholesterol-stabilizing effect of the phospholipids. It would be of particular interest to study the effect on that spectrum of lipoproteins which represent a gap between the lipoprotein macromolecules of high flotation rates

(about S_f 400 and higher) and the smallest (about 0.1 micron) microscopically visible particles. Unfortunately there are considerable technical difficulties connected with the study of that intermediary segment of the lipoproteins.

The preliminary observations reported here not only should receive long-term clinical evaluation, but should also be extended by further studies utilizing all known biochemical and biophysical approaches to supplement each bit of additional knowledge by a broad, unbiased, and detached approach to this perplexing problem.

Summary

Lipotropic substances—choline, methionine, and inositol in a balanced formula (Methischol)—were administered to 11 patients with proved past myocardial infarction for not less than nine weeks and to 24 elderly women for nine weeks, and to 12 for twelve weeks, the latter group having no history of myocardial infarction.

After nine or more weeks of administration of the lipotropic mixture to the myocardial infarction group, there was a decrease of borderline statistical significance in the total serum cholesterol levels. No such variation was observed in the control group even after prolonged therapy; neither was there any appreciable variation following the administration of a lactose placebo in capsules of identical appearance.

There was a statistically highly significant depression of chylomicron levels (per cent of microscopically visible lipoprotein particles 0.3 micron in diameter and larger) in all subjects on the above regime. Intermittent discontinuation and repeated administration of these substances in four atherosclerotic subjects, followed from five to twelve months, resulted alternately in elevation and depression of chylomicron levels, respectively. Placebo administration did not result in a change in chylomicron levels beyond the statistically expected and experimentally confirmed variation.

Addendum

Since our original data were tabulated and statistically analyzed we have maintained 18 more patients with clinically demonstrable coronary atherosclerosis on lipotropic therapy. The mean chylomicron levels for that group of 18 patients, on the basis of repeated tests prior to any medication, was 44.96 per cent; all patients without exception showed the same trend manifested by the subjects in the original study. The mean chylomicron levels at the end of lipotropic therapy was 31.29 per cent. The shortest period of administration was 15 weeks; the longest period, 54 weeks; the average was 29 weeks. It is of interest that discontinuation of therapy for a certain period of time or decrease in the daily dose resulted in the increase of the fasting chylomicron levels; resumption of therapy or increase in the dose resulted in the lowering of the fasting chylomicron levels. Our observation on the effect of lipotropic therapy recently received confirmation by another group.[14]

REFERENCES

1. Pederson, K. O.: *Ultracentrifugal Studies on Serum and Serum Fractions.* Almqvist and Wiksells Boktryckeri, Upsala, 1945.
2. Frazer, A. C., and Stewart, H. C.: Ultramicroscopic particles in normal human blood. *J. Physiol.* 90: 18, 1937.
3. Elkes, J. J., Frazer, A. C., and Stewart, H. C.: Composition of particles seen in normal human blood under dark-ground illumination. *J. Physiol.* 95: 68, 1939.
4. Steiner, A.: Symposium on cardiovascular diseases; cholesterol in arteriosclerosis, with special reference to coronary arteriosclerosis. *Med. Clin. N. Am.* 34: 673, 1950.
5. Morrison, L. N., Hall, L., and Chaney, A. L.: Cholesterol metabolism: blood serum cholesterol and ester levels in 200 cases of acute coronary thrombosis. *Am. J. M. Sc.* 216: 32, 1948.
6. Zinn, W. H., and Griffith, G. C.: Study of serum fat globules in atherosclerotic and non-atherosclerotic male subjects. *Am. J. M. Sc.* 220: 597, 1950.
7. Labecki, T. D.: A study of serum lipids in atherosclerotic and apparently healthy subjects. *J. Gerontol.* 7: 484, 1952.
8. Labecki, T. D.: Diagnostic value of serum cholesterol determination, ultracentrifuge studies

and chylomicron levels in fasting serum. *Circulation* 8: 446, 1953.

9. LABECKI, T. D.: Hyperchylomicronemia and hypercholesterolemia: their correlation with clinical atherosclerosis. AM. J. CLIN. NUTRITION 3: 132, 1955.
10. BEST, C. H., LUCAS, C. C., PATTERSON, J. N., and RIDOUT, J. H.: Lipotropic properties of inositol. *Science* 103: 12, 1946.
11. GOLDSTEIN, M. R., and ROSAHN, P. D.: Choline and inositol therapy of cirrhosis of the liver. *Connecticut M. J.* 9: 351, 1945.
12. DOLAN, R. A.: Choline and other lipotropic factors; mechanism of action and significance in chronic liver disease. *Minnesota Med.* 31: 1198, 1948.
13. FOA, C.: Protection of the liver and the kidneys. "Foreign Letters," *J.A.M.A.* 134: 1041, 1947.
14. RAWLS, W. R.: Personal communication.

Avoiding Osteoporosis

"In the ballet, age is usually depicted with the tremor and festinant gait of Parkinsonism, the ineptitude and labile mood of an early senile dementia, and the painful, bent back of senile osteoporosis. And the artist is right, in stressing the trouble that ageing bone can cause. . . . If we could bring our patients into old age with strong bones we would save them a vast amount of suffering and the country a great deal of money. . . .

On the one hand, not enough osteoid tissue may be formed: on the other, calcium may be deposited in it in inadequate amounts or removed from it too fast. The first pathological change is called osteoporosis, and the second osteomalacia.

"Nature has not ordained that patients should pay close attention to this classification, and many of them, particularly the elderly, have a combination of conditions.

"We have. . .as some of the known and suspected causes why many old people have brittle bones: (1) An imbalance of hormones. (2) A diet deficient in calcium, protein, vitamin D, and, perhaps, other essential factors. (3) Deficient absorption of calcium. (4) Inactivity, or even immobility.

"At least three of these causes are preventable: all are treatable. . . . We do not know how to keep elderly people 'in endocrine balance.' We can only attempt to diagnose the condition and treat it early in its course. . . . On the other hand, we can ensure an adequate intake of calcium.

"Our greatest endeavours must. . .be directed toward. . .prevention, or, failing that, . . . early detection and treatment. As people grow old, they should keep as active as possible, and take a diet containing adequate amounts of calcium, vitamin D, and protein."

—J. R. Bolton. *The Medical Press* 232: 381–385, 1954.

Editorial

Human Pantothenic Acid Deficiency

Pantothenic acid deficiency has been experimentally induced in man by Bean and Hodges.[1] They administered a diet deficient in this factor together with a metabolic antagonist (omega-methyl pantothenic acid) to four volunteers.

A number of interesting clinical and laboratory changes were noted. Among these were postural hypotension, fatigability, and, later, epigastric distress, numbness and paresthesias of the hands and feet, hyperreflexia, and emotional changes. Despite a dietary intake of more than 1000 calories and 100 Gm. protein daily, weight loss was progressive. An increased susceptibility to respiratory infections was noted. Impaired acetylation and impaired adrenal function, gastric hypochlorhydria, and a fall in blood cholesterol levels were also found.

Pantothenic acid alone was then added and although the paresthesias improved promptly, the urinary 17-ketosteroid excretion, already depressed, decreased further. It became clearly evident that the clinical manifestations of the deficiency were progressing; in fact, rather alarming symptoms developed which were suggestive of adrenal insufficiency, and cortisone treatment was necessary. A rapid and complete recovery in clinical and laboratory spheres followed administration of a *general diet* supplemented with oral and parenteral vitamins.

As the authors note, spontaneous development of such a deficiency syndrome is highly unlikely because of the wide distribution of the vitamin in foods and, unlike pyridoxine, because of its resistance to destruction by heat or chemicals. Nevertheless, this is an important subject because pantothenic acid is a portion of that remarkable and crucial substance, coenzyme A. Some of the signs and symptoms and biochemical alterations in this syndrome are undoubtedly due to impaired acetylation —a metabolic process dependent on an intact coenzyme A mechanism.

It is possible that the metabolic antagonist may have had some toxic properties of its own,[2] because a favorable response was not induced by pantothenic acid alone. It is equally possible that the deficiency was present for so long that progressive bodily changes were developing which could not be simply reversed by pantothenic acid supplementation. It is perhaps more likely that the diet plus antagonist produced a deficiency of more than the single vitamin (perhaps an as yet unknown factor), so that it took a general mixed diet in addition to vitamin B complex supplementation to bring about a cure.

These observations are of considerable interest and suggest that investigators and clinicians will be hearing much more about pantothenic acid.

—S. O. Waife, M.D.

REFERENCES

1. Bean, W. B., and Hodges, R. E.: Pantothenic acid deficiency induced in human subjects. *Proc. Soc. Exper. Biol. & Med.* 86: 693, 1954.
2. Induced pantothenic acid deficiency in man. *Nutrition Rev.* 13: 36, 1955.

Nutritional Etymology

For those who like the play of words, medical etymology offers a brief respite from the stresses and alarm reactions of every day.

Nutritional terms have interesting derivations, often symbolizing the connection of nutrients and life.

NUTRITION comes from the Latin word *nutrire,* to suckle or feed—the primary act of suckling so characteristic of the process of nutrition.

METABOLISM is derived from the Greek *metabole,* a change. Not only is metabolism a changing process, but the end-product is a METABOLITE.[1]

The derivations of some words are classical in their simplicity. Thus, CARBOHYDRATE comes from *carbo,* Latin for coal, i.e. carbon, and the Greek *hydor,* water. (Greek and Latin hybridization occurs frequently in medicine [e.g. glomerulonephritis, autoclave, dextrocardia, etc.] and, although anathema to the purists, such terms are here to stay—an indication of the perverse imperfection of man.)

PROTEIN is logically a derivative of the Greek word for "primary," *proteios;* while LIPID is clearly from *lipos,* the word for fat in Greek.

Our term DIET is a modification of the Greek *diaita,* a way of living or regimen. As the word spread in the days of the Romans and French its meaning became restricted. Curiously we say "dietary regimen."

The vitamins are named with a certain degree of logic. The structure of THIAMINE is responsible for its name. *Theion* is the Greek word for sulfur; the AMINE is a term selected a century ago for "substances derived from ammonia."[1]

RIBOFLAVIN has the RIBO from a coined word (ribonic acid), and FLAVIN from *flavus,* Latin for yellow.

Since scurvy is *scorbutus* in Latin, ASCORBIC ACID was created by adding the Greek *a* for "not."

RICKETS has an uncertain origin. The word for an inflammation of the spine, *rachitis* (Greek *rachis,* spine) was applied originally to hump-back, but now is used for the vitamin D deficiency state. There is also an Old English term, *rick,* which means twist; either way the twisted spine was clearly in the minds of the word-coiners.

Obdere means to "eat up" in Latin; from this comes *obesus,* fat, and our all-too-common word OBESITY.

PELLAGRA could have two derivations: from Italian, where *pelle* means skin, and *agra* rough; or from Latin *pellis,* skin, and *agra,* Germanic for seizure or disease.

That honorable element IRON has its variations. In Old English *iren* meant iron, and the change in spelling dates only from 300 years ago. The Greek term, *sideros,* appears in SIDEROSIS, while the Latin term, *ferrum,* appears as FERROUS and its variations.

So much for looking backward. It is to be hoped that medical terms yet unborn will fulfill the requirements of accuracy, clarity, and

—S. O. WAIFE, M.D.

REFERENCE

1. Pepper, O. H. P.: *Medical Etymology,* W. B. Saunders, Philadelphia, 1949.

Dietotherapy

NUTRITION AND THE PRESCHOOL CHILD

By LORRAINE WENG*

MODERN NUTRITIONAL concepts emphasize that professional workers outlining feeding programs for young children must consider two objectives: (1) to provide food which will supply the nutrients required for growth and well-being; and (2) to suggest methods which will guide children toward the proper selection of food throughout life. This approach has resulted from the experiences of physicians and others in trying to correct feeding problems in young children. Studies of established cases of such problems suggest that the following may be of particular significance in their etiology:

1. Failure to recognize that rate of physical growth may affect appetite.
2. Failure to recognize that personality and emotional development may affect appetite.
3. Insisting that specific amounts of food be eaten without regard for day-to-day variation or individual preferences.
4. Failure to respect individual differences in body size as influencing dietary needs.
5. The emotional interplay between the adults in the family (particularly the mother) and the child.
6. Lack of consistency in methods of feeding the child.
7. Failure to recognize loss of appetite associated with illness and convalescence.
8. Overanxiety and misunderstanding of the baby's first reaction to solid food, his interest in self feeding, and his need to learn about adult food.
9. Misunderstanding of how a child's food preferences, the type of eating utensils provided, the content of the day's meal, between-meal feeding, and food aversions of other members of the family affect his appetite.

The late Dr. C. A. Aldrich[1] was particularly interested in this problem and is often credited with doing much of the pioneer work toward helping parents and professional people understand the child's mechanism for getting food. He pointed out that "if we are to have any success in solving feeding difficulties, we cannot think only in terms of diets; we must also know something about a child's response to food, his ability to 'come and get it.' We must accept the fact that he has a feeding mechanism of his own, which is designed to make him a satisfactory and capable eater, and must understand how this feeding ability works."

This twofold approach is stressed because it has so frequently been minimized. Certainly the question of "what" to feed children is of the utmost importance and it is our chief concern here. However, we must always be cognizant that general guides are helpful when applied to groups of children, but they may need adaptation when applied to individuals.

TABLE I

Recommended Dietary Allowances for Children 1 through 6 years

	1–3 years	4–6 years
Calories	1200	1600
Protein (Gm.)	40	50
Calcium (Gm.)	1.0	1.0
Iron (mg.)	7	8
Vitamin A (I.U.)	2000	2500
Thiamine (mg.)	0.6	0.8
Riboflavin (mg.)	1.0	1.2
Niacin (mg.)	6	8
Ascorbic Acid (mg.)	35	50
Vitamin D (I.U.)	400	400

* National Dairy Council, 111 North Canal Street, Chicago 6, Ill.

TABLE II

A Daily Guide for Feeding Children From 1 to 6 Years of Age*

Food group	Amount recommended	Average size serving		
		1 year	*2–3 years*	*4–6 years*
Milk	3–4 measuring cups	1 cup at each meal may be supplemented by milk between meals		
Meat, Poultry, or Fish	1 to 2 ounces	1 ounce (2 tablespoons)	1½ ounces (3 tablespoons)	2 ounces (4 tablespoons)
Egg	1 egg		1 egg	
Fruit				
Citrus or tomato	1 medium orange, ⅓–½ cup of orange or grapefruit juice, or ¾ to 1 cup tomato juice	Whole day's amount in 1–2 servings		
Other	1–2 servings	¼ cup	⅓ cup	½ cup
Vegetables				
Cooked	1–2 servings	2 tablespoons	3 tablespoons	4–5 tablespoons
Raw (carrots, celery, lettuce)	....		1 or 2 small pieces	
Bread and Cereal (Wholegrain or enriched)				
Bread	1½–3 slices	½–1 slice	1–1½ slices	1½–3 slices
Cereal	¼–½ cup	¼ cup	⅓ cup	½ cup
Butter	1 to 2 tablespoons	1 teaspoon	1–3 teaspoons	3–6 teaspoons
Source of Vitamin D	400 I.U.	Vitamin D milk or a preparation prescribed by the physician		

* Adapted from chart, Foods Included in a Good Daily Diet for Children from 1 to 6, in *Your Child from One to Six,* Rev. Ed., U. S .Children's Bureau Pub. No. 30, 1945.

The Recommended Daily Dietary Allowances[2] for preschool children proposed by the Food and Nutrition Board of the National Research Council are given in Table I.

These "allowances are designed for the maintenance of good nutrition of healthy persons in the United States under present conditions. . . . The nutritive intakes recommended are, in general, higher than average requirements and lower than the amounts which may be needed in pathologic states or in rehabilitation following depletion." In planning dietaries for children, it is suggested that the food needs of healthy children be considered first, and then that modifications be made for special circumstances such as illness or hospitalization.

Table II summarizes the major food groups that should be included in meal plans for the preschool child, amounts recommended, and the average size serving that children from one to six years may be expected to eat. When this guide is followed, it is reasonable to assume that the Recommended Daily Dietary Allowances for specific nutrients will also be met.

These foods can be arranged into meals by following a meal pattern something like this:

Meal Pattern	Sample Menu
Breakfast	
Citrus fruit	Orange Juice
Wholegrain or enriched cereal	Oatmeal
Wholegrain or enriched bread	Toast and butter
Butter	Milk
Milk	
Lunch or Supper	
Egg or Cheese	Cheese Sandwich
Vegetable	Carrot Sticks
Wholegrain or enriched bread	Vanilla Pudding
Butter	Milk
Dessert	
Milk	

Meal Pattern	Sample Menu
Dinner	
Meat, Poultry, or Fish	Beef Patty
Potato	Mashed Potato
Vegetable	Buttered Green Beans
Wholegrain or enriched bread	Bread and Butter
Butter	Sliced peaches
Fruit	Milk
Milk	

It is extremely important to recognize that a pattern such as the one given is adaptable. We have become accustomed to thinking of "orange juice for breakfast," "a sandwich for lunch," "green vegetables for children," and yet such concepts may actually limit food selection. There is no reason why orange juice cannot be given at a time other than breakfast or why an extra slice of toast cannot be substituted for cereal. Thinking in terms of food groups and interchangeability of foods within the group can contribute to flexibility in menu planning.

It may be advantageous to consider the recommended food groups in somewhat greater detail.

Milk and Milk Products

Three to four cups of milk or the equivalent will help to supply the quantities of calcium, protein, vitamin A, riboflavin, and thiamine recommended by the Food and Nutrition Board. In localities where Vitamin D milk is available, the milk will contribute to the suggested allowance of 400 I.U. vitamin D per day.

It is difficult to plan diets that meet the Recommended Daily Dietary Allowances without including milk. However, young children sometimes do go through periods when they cannot, or will not, take as much as 3 or 4 cups of milk a day. Temporarily reducing the quantity of milk offered is wiser than creating an issue. Cheese made from whole milk, such as cheddar cheese, is a satisfactory substitute for the milk and is usually well accepted. Cottage cheese is a good source of protein but is not as high in calcium and some vitamins.

Meat, Poultry, or Fish

The main contribution of this food group to the diet is the protein, iron, thiamine, riboflavin, and niacin it supplies.

Tender, soft meats are popular with young children. Cooking temperatures are important because protein foods such as meat, poultry, and fish toughen when they are cooked at high temperatures. Less tender cuts of meat can be ground before cooking and made into patties or loaves. Manipulation of a spoon or fork requires a high degree of muscle coordination which develops as the child grows. During his early years, however, it is easier for him to eat with his hands than it is to use an eating utensil. Bite-size pieces of meat are easy to manage and help the child learn to feed himself, and also let him learn about food through feeling it.

Eggs

One egg a day along with the recommended amounts of milk, meat, poultry, or fish will supply sufficient protein for the preschool child. Eggs are also an important source of iron, niacin, riboflavin, and thiamine.

For variety, eggs can be cooked in different ways—baked, poached, scrambled, soft-cooked, or hard-cooked and served as a "finger food." As with meat, high temperatures will toughen eggs. Therefore, they should be cooked at a temperature below boiling.

Fruits

The Recommended Daily Dietary Allowance of 35–50 mg. ascorbic acid for children between 1 and 6 years can be satisfied by an orange, $^1/_2$ grapefruit, $^1/_3$–$^1/_2$ cup orange or grapefruit juice, or $^3/_4$ to 1 cup tomato juice. It can also be satisfied with a combination of other fruits such as berries and melon.

Other fruits—bananas, apples, pears, peaches, prunes, apricots, and so on—will help to meet the mineral and vitamin needs of the body, especially for iron, thiamine, and riboflavin. The yellow fruits will also contribute vitamin A.

Apples, peaches, bananas, and oranges can be peeled and served raw. Seeds should be

carefully removed so there is no danger of aspiration.

Vegetables

Vegetables in general are valuable for roughage, minerals, and vitamins. The green leafy group is particularly important for its iron and vitamins A and C. The yellow group of vegetables is valuable for its vitamin A. Dried beans and peas contribute protein and B-complex vitamins as well as energy. Potatoes make a general contribution of minerals and vitamins as well as of energy.

Raw vegetables are excellent finger foods—carrot sticks, celery sticks, wedges of lettuce or cabbage—and they are generally popular with young children. In fact, raw vegetables will often be eaten with relish, while the same vegetables cooked may be refused.

Some children go through stages when they refuse to eat vegetables altogether. As with milk, it is more desirable to reduce or eliminate the vegetables for awhile than it is to urge and push the child to eat them. Extra servings of fruit can be served, since they will supply essentially the same nutrients.

Bread and Cereal

Wholegrain or enriched bread and cereals are important sources of energy, and contribute niacin, thiamine, riboflavin, and iron. They also furnish significant amounts of protein.

In cooking cereals, it may be necessary to add extra liquid so that they do not become thick and dry when cooled.

Butter

Butter has two important contributions to make toward fulfilling the food needs of preschool children—its vitamin A and its energy value. It will also help to improve the flavor of cooked vegetables.

One of the reasons that it is difficult for many people to allow children some leeway in eating is the fear they will not eat "enough" or the "right" kind of food. "Is there a specific mechanism for food selection based on body need?" Controlled experiments with human subjects attempting to answer this question are few. The one most widely quoted was planned and directed by Dr. Clara Davis[3,4] with newly weaned infants. Her original research included three infants, and it was later extended to include an additional fifteen. The children were fed "a large assortment of cooked and raw, uncombined, unseasoned, and unsophisticated foods according to a schedule of three meals a day, and no food given between meals. Each child had his own small table and the foods for the meal were set before him on a tray. The children ate with fingers or spoons, depending on ability or preference, and empty dishes were promptly refilled to make sure that the children got all they wanted of everything. Food and eating were never discussed by the attendants in the presence of the children." In this setting, "anorexia, other than as a transient loss of appetite with acute parenteral infection, was unknown among them, all eating heartily with keen appetite day in, day out. It was not unusual for children to eat 3 or 4 bananas at a meal or 5–7 servings of potatoes or meat. One child a year old frequently drank a quart of milk at his noon meal besides eating other food. Yet none was fat, and all were solid well built children, healthy and vigorous, presenting no problems in behavior. A few instances of vomiting or diarrhea occurred with an acute parenteral infection, but otherwise there were no digestive disturbances and never any constipation, laxatives never being needed or given to any. The study demonstrated that under the conditions of the experiment and with such natural foodstuffs young children could choose their diets and thrive without adult direction of eating." This was a small number of cases and Dr. Davis has emphasized that (1) this was a group of children who had not been indoctrinated with any previous "dietary information or experience by either example or instruction," and (2) this experiment did not test the ability of the child to choose between natural foods and highly refined or processed foods. The evidence does not prove conclusively that appetite can function as an indicator of nutritional needs. However, the experiment does show that under certain conditions, children can be allowed

to exercise some freedom of choice at least.

Dr. Miriam Lowenberg[5] has given the following suggestions as guides to fostering good eating habits in children.

1. See that the children get enough rest and relaxation, so they will not be tired at mealtime.

2. Try to have a cheerful and happy atmosphere at mealtime.

3. See that the child is seated comfortably.

4. Provide dishes and utensils that are suitable for small hands.

5. Prepare for spilling and other messy eating by providing bibs and table protectors. Don't expect little children to eat as skillfully as adults.

6. Prepare foods so they are attractive to children and not too difficult to manage.

7. Serve small portions, and second helpings when needed. Remember that portions that are too large discourage a child from eating.

8. Expect children to eat. Remember that they readily sense the attitude that the adults who eat with them show toward their food.

9. Remember that each child is an individual. Do not expect all children, even of the same age, to have the same tastes in food, nor to eat the same amounts.

10. Encourage interesting and pleasant table conversation among children who are old enough to eat well and to talk at the same time. Talking about personal likes and dislikes for food should be discouraged. The foods themselves, and where they come from, are usually interesting for children to talk about.

11. Remember that courtesy at the table is not dependent on "thank you" and "please." Do not expect adult politeness of little children. If they are with adults who are innately courteous, they will pick up socially acceptable manners after they have mastered the difficult task of feeding themselves. But do not expect them to do both at once.

To summarize, pleasant eating experiences as well as proper foods are important to the successful feeding of children.

REFERENCES

1. Aldrich, C. A., and Aldrich, M.: *Feeding Our Old Fashioned Children.* The Macmillan Company, New York, 1947.
2. Food and Nutrition Board: *Recommended Dietary Allowances Revised, 1953.* Washington, D. C.
3. Davis, C. M.: Self selection of diet by newly weaned infants. *Am. J. Dis. Child.* 36: 651, 1928.
4. Davis, C. M.: Feeding after the first year. In *Practice of Pediatrics,* Vol. 1. (Brennemann, J. E., ed.), W. T. Prior Co., Inc., Hagerstown, Md., 1942, chap. 30.
5. Lowenberg, M. E.: Food for Young Children, in *Group Care,* Rev. Ed., U. S. Children's Bureau Pub. No. 285, 1947.

Nutrition News

☆ *News of activities in the field of clinical nutrition should be submitted to the Editorial Office of the* JOURNAL.

Symposium on Antimetabolites

A symposium on *The Antimetabolites—Their Modes of Action and Therapeutic Implications,* under the double chairmanship of Dr. Paul L. Day (University of Arkansas) and Dr. R. W. Heinle (The Upjohn Company), was held in New York City on March 1, 1955, under the sponsorship of the National Vitamin Foundation.

The following papers were read:

Anti-Vitamin E Stress Factors as Related to Fatty Peroxides (E. L. Hove).

Thiamine Antagonists (L. R. Cerecedo).

Vitamin K Antagonists (K. P. Link).

Folic Acid Antagonists (J. H. Burchenal).

Purine and Pyramidine Antagonists (G. H. Hitchings).

A Naturally Occurring Antimetabolite of Methionine in the Causation of a Disease (D. W. Woolley).

Pantothenic Acid Antagonists (O. D. Bird).

Vitamin B_6 Antagonists (W. W. Unbreit).

Riboflavin Antagonists (J. P. Lambooy).

The proceedings of this Symposium will be published as a special number of The AMERICAN JOURNAL OF CLINICAL NUTRITION.

Sir Edward Mellanby

We learn with regret of the recent death, in London, of Sir Edward Mellanby, distinguished British nutritionist, discoverer of vitamin D, and enemy of Agene. Sir Edward's death, at 70, was due to coronary thrombosis.

While the use of Agene as a bleaching and aging agent has been banned by the U. S. Food and Drug Administration, and was condemned by the British Government in 1950, Sir Edward did not live to see its actual discontinuance as an "improver" of British bread. However, the deadline for its disappearance has been fixed by the National Association of British and Irish Millers for December 31, 1955.

Sir Edward was associated with this JOURNAL from the start, as a Corresponding Editor; it will be difficult to replace him.

Nutritional Quotes

Steroid Synthesis in Pantothenic Acid Deficiency

"The following hypothesis is therefore postulated: in the normal animal subjected to stress, cholesterol is used in the synthesis of adrenal steroids, with coenzyme A as a necessary component of the system. In the pantothenic acid-deficient animal, the synthesis of cortical steroids is begun, but in the absence of adequate amounts of coenzyme A the process cannot be carried to completion and the synthesis stops at some intermediate step. The compound or compounds elaborated are therefore not effective for gluconeogenesis. Such a hypothesis . . . would explain the fate of cholesterol which had disappeared from the adrenal glands of pantothenic acid-deficient animals in spite of the absence of increased gluconeogenesis."

—L. S. Hurley and J. B. Mackenzie. *Journal of Nutrition* 54: 403, 1954.

Postgastrectomy Avitaminosis

"We have observed clinical evidence of riboflavin deficiency in five patients of the present series (75 subjects 10 years after partial gastrectomy) but have not detected any true evidence of deficiency of other factors of the B group. It is curious that the patients suffering from riboflavin deficiency were not those who showed evidence of loss of weight; indeed, two excelled their original 'health' weight. This demonstrates a selective absorption deficiency which is not constant for all dietary articles. Our previous evidence of avitaminosis-B (*Lancet* 1: 939, 1951) suggested that it occurred in about 10 percent of all patients who have undergone gastrectomy. This figure is substantiated by the results of the present series. Further, we have no evidence to show that within limits the extent of gastric resection has any bearing on the development of avitaminosis-B."

—C. Wells and I. W. MacPhee. *British Medical Journal* (November 13, 1954), p. 1128.

"Glucostasis"

"The problem of appetite and its relation to obesity has been approached by Mayer and his colleagues. They have proposed a 'glucostatic theory' of the regulation of food intake, which depends upon the degree of arteriovenous glucose difference. In essence, appetite is thought to be associated with a small arteriovenous glucose difference and anorexia with a greater one. In one of their studies, 12 obese and 12 control women were studied with respect to glucose tolerance after the ingestion of an ordinary meal and after glucose by mouth. Strikingly different curves were obtained, the obese persons having a considerably greater tolerance both to carbohydrate from the meal and to glucose. When dextrose was administered intravenously, a similarly increased tolerance was observed in the obese persons. The increased carbohydrate tolerance in these persons presumably leads to a more rapid disappearance of the arteriovenous difference seen after glucose ingestion. This disappearance of the arteriovenous difference has been termed by Mayer and his colleagues 'metabolic hypoglycemia' and in itself is thought to lead to hunger. 'Active' obese persons tend to have a period of metabolic hypoglycemia sooner than persons who maintain a normal weight, that is, 'satiety is more transient.'

"Finally, these workers were able to show that arteriovenous glucose correlated closely with appetite and food intake. It was found that an arteriovenous glucose difference of 15 mg. per 100 cc. or greater was never associated with hunger, whereas values approaching zero 'were invariably associated with hunger.' The results collected and interpreted by these workers form an important approach to the study and treatment of obesity in man."

—Charles S. Davidson. *A. M. A. Archives of Internal Medicine* 94: 463–464, 1954.

Iodine Requirements

"Balance studies have indicated that the requirement for iodine is probably about 0.002 to 0.004 milligrams daily for each kilogram of body weight. The need for iodine is increased during adolescence in girls, during pregnancy, and probably during growth. . . .

"These figures indicate an average requirement of 100 to 300 micrograms daily for an adult, depending upon body weight. . . .

"There are, however, a number of factors tending to produce goiter by increasing the relative requirements for iodine. Among these are compounds present in cabbage, kale, brussel-sprouts, ground-nuts, soya, and other foodstuffs. The ingestion of an excessive amount of calcium can increase the iodine requirement, as can also deficiency of vitamin A. There are also other factors, still unidentified, which have the same effect."

—*Chronicles of the World Health Organization* 8: 320, 1954.

Amino Acids and Calories

"With mixtures of purified amino acid, each of the three subjects experienced a loss of body nitrogen until his caloric intake was increased. . . . In a

single experiment, no difference could be detected in the metabolic behavior of the subject when he received a mixture containing all of the amino acids recognized as components of casein as contrasted with one carrying the eight essentials supplemented with additional sources of nitrogen. . . .

"The authors are concerned primarily with comparisons of whole casein and mixtures of purified amino acids, since the latter have been used throughout the entire program dealing with the amino acid requirements of man. The data herein recorded appear to leave no reasonable grounds for doubting that diets containing such amino acid mixtures necessitate higher caloric intakes for nitrogen balance than do like rations containing whole casein. No satisfactory explanation can be offered to account for these unexpected observations. One might anticipate that amino acids would be absorbed from the alimentary tract more rapidly when ingested in the free state than when they must first be liberated from proteins by the process of digestion. Conceivably, this might lead to a temporary flooding of the organism, thereby inducing either a spillage into the urine or an accelerated catabolism. That wastage through excretion does not occur to a significant extent is demonstrated by the daily determinations of urinary α-amino nitrogen as summarized in the preceding papers of this series. . . . The possibility that an accelerated catabolism of the free amino acids may have occurred cannot be excluded, but, in that event, one might more reasonably expect the organism to require an increased nitrogen intake rather than a higher energy consumption."

—W. C. Rose, M. J. Coon, and G. F. Lambert. *The Journal of Biological Chemistry* 210: 340–341, 1954.

Complications of Sodium Restriction

"The restriction of sodium intake, although of definite therapeutic value in certain diseases, is not without its harmful effects and even dangers. Prolonged sodium restriction apparently does no harm if the renal and sweat mechanisms which normally maximally conserve sodium in this circumstance are intact. Many persons with congestive heart failure and, particularly, some patients with chronic nephritis have a greatly reduced effectiveness of the renal mechanism for salt retention, so that sodium continues to be excreted in the urine (and sweat) even when the intake in food is reduced to a very low level. The same situation may be induced in certain patients by the repeated administration of mercurial diuretics, particularly when the sodium intake is greatly restricted. These circumstances lead to a body depletion of sodium which makes itself known by oliguria, continued or increased edema formation, extrarenal azotemia, cramps in the extremities, and even a fatal outcome. This syndrome in its acute stages can be readily treated by the intravenous administration of hypertonic saline solution and by restriction of water intake. When the state of sodium depletion is chronic, however, it may be more difficult to recognize but still may play an important part of the genesis of weakness and general debility in certain patients. It should be emphasized again, however, that most patients have normal homeostatic mechanisms and are able to manage well even severe sodium restriction and benefit by the associated control of edema and ascites formation."

—Charles S. Davidson. *A. M. A. Archives of Internal Medicine* 94: 467–468, 1954.

Nutrition Briefs

CURRENT OBSERVATIONS OF CLINICAL INTEREST

MORE THAN 20 per cent of the population of the United States is overweight. From 21 to 27 per cent weigh more than 10 per cent above "standard or desirable" weight. From 7 to 12 per cent of the population is 20 per cent or more overweight.

Pub. Health Rep. 69: 1084, 1954.

DIURNAL variations in serum iron level are independent of eating, exercise, body temperature, or blood pH. Evidence suggests that the serum level is dependent on erythropoietic activity and implies that hemoglobin synthesis is slowed down during sleep.

J. C. S. Paterson. *J. Lab. & Clin. Med.* 44: 906, 1954.

PERIPHERAL neuritis may occur in as many as 40 per cent of patients receiving isoniazid for tuberculosis. This drug causes an excess excretion of pyridoxine. When pyridoxine is administered with isoniazid, peripheral neuritis is prevented.

J. P. Biehl and R. W. Vilter. *J. A. M. A.* 156: 1549, 1954.

PATIENTS with cirrhosis, hepatitis, and fatty liver were given a high fat diet (300 Gm. fat, 100–120 Gm. carbohydrate, 4000 or more calories). The patients improved; no alterations in blood lipids were found and gastrointestinal disturbances were mild.

G. Mindrum. *J. Lab. & Clin. Med.* 44: 898, 1954.

INTRAVENOUS infusions of Tween 60® (polyoxyethylene sorbitan monostearate) fat emulsions in man and animals led to numerous acute side effects.

J. L. Schmidt, I. H. Strub, R. K. Richards, M. I. Grossman, and H. D. Brondyk. *J. Lab. & Clin. Med.* 44: 926, 1954.

NO EFFECT on blood clotting time, on *in vitro* "heparin tolerance," or on platelet count was noted following the ingestion of a fatty meal. This contradicts previous reports, which may have been erroneous because of faulty techniques.

R. C. Haitmann, C. B. Thorne, and H. C. Meng. *Clin. Research Proc.* 3: 68, 1955.

LOW SODIUM diets plus Carbo-Resin® were given for from one to three years to outpatients with severe hypertension. The 24-hour urine solution excretion was kept at below 5 mEq; and significant objective and subjective improvement was noted.

B. L. Martz. *J. Lab. & Clin. Med.* 44: 896, 1954.

SODIUM salicylate—unlike *p*-amino salicylic acid, which decreases urinary elimination of thiamine—had no effect on thiamine excretion when administered orally to humans.

M. Ramunni. *Acta vitaminol.* 8: 164, 1954.

TWO PATIENTS with pernicious anemia each received one intranasal application of crystalline vitamin B_{12} (150 and 200 μg.). An excellent reticulocyte and hematologic response was obtained, with remissions lasting two and three months. The crystals are nonirritating, and absorption is probably not dependent upon binding with intrinsic factor.

R. W. Monto, J. W. Rebuck, and J. T. Howell. *J. Lab. & Clin. Med.* 44: 900, 1954.

WEEKLY oral doses of 1 mg. (1000 mcg.) of vitamin B_{12} without intrinsic factor material seem to provide adequate therapy for most patients with pernicious anemia.

E. H. Reisner, Jr., L. Weiner, M. T. Schittone, and E. A. Henck. *Clin. Research Proc.* 3: 28, 1955.

DAILY oral administration of 25 μg. vitamin B_{12} with 1.67 mg. folic acid is inadequate for the maintenance of the patient with pernicious anemia.

S. O. Schwartz, I. A. Friedman, and H. L. Gant. *J. Lab. & Clin. Med.* 44: 929, 1954.

RECENT ADVANCES IN EXPERIMENTAL NUTRITION

SERUM glucoproteins were studied in guinea-pigs with experimental avitaminosis C. There was a significant increase in perchloride-soluble mucoproteins, total protein polysaccharides, and glucosamines bound to total serum proteins. This increase is interpreted on the basis of a depolymerization of the mucopolysaccharides of the connective tissue ground substance in scurvy.

E. Bonomo and M. Chirico. *Acta vitaminol.* 8: 173, 1954.

THE CARCINOGENIC action of 3-4-benzopyrene on white rats was enhanced by the oral administration of unsaturated fatty acids, which shortened the latent period of the carcinogenicity.

G. De Angelis, W. Ciusa, and G. Nebbia. *Acta vitaminol.* 8: 161, 1954.

SEVERAL related compounds used in experimental cancer therapy (6-mercaptopurine, azaserine, etc.) affect tryptophane metabolism. Simultaneous pyridoxine administration inhibits this metabolic abnormality.

J. W. Magill, G. B. Magill, and J. H. Burchenal. *Clin. Research Proc.* 3: 21, 1955.

DIETARY hepatic cirrhosis can be induced in rats by feeding a ration with Torula yeast as sole source of protein. The diet is free of vitamin E, and deficient in cystine. Biochemical defects were corrected and necrosis prevented by administration of vitamin E.

M. Rosecan, G. P. Rodnam, S. C. Chernick, and K. Schwarz. *J. Lab. & Clin. Med.* 44: 919, 1954.

PUPPIES, 3 months old, had bile diverted through the kidneys. Rapid and severe hypoprothrombinemia developed in spite of parenteral vitamin K, as well as A and D. No noticeable effects were seen on the digestive or skeletal systems. The animals matured normally.

A. J. Quick, C. V. Hussey, and G. E. Collentine, Jr. *J. Lab. & Clin. Med.* 44: 909, 1954.

PANTOYLTAURINE has been shown to be an antivitamin for pantothenic acid. By including pantoyltaurine in the diet of pregnant rats, various congenital malformations were produced, particularly dehiscence of the cerebral substance, edema, and hemorrhage. These anomalies are the same as those achieved with diets deficient in pantothenic acid.

C. Zunin and C. Borrone. *Acta vitaminol.* 8: 263, 1954.

AN ANALOGUE of riboflavin was found to be a reversible antagonist of the vitamin. It also produced significant granulocytosis without lymphopenia. There is evidence that the analogue interferes with vital processes of certain malignant cells.

E.-A. Musser, H. G. Petering, and R. W. Heinle. *J. Lab. & Clin. Med.* 44: 903, 1954.

A PROTECTIVE action is claimed for vitamin E (alpha-tocopherol) in experimental subacute lead poisoning in rabbits. Coproporphynuria and anemia were less severe in animals treated with vitamin E than in untreated controls.

R. de Rosa. *Acta vitaminol.* 8: 167, 1954.

A SUBSTANCE in licorice (glycyrrhizinic acid) produces large retention of sodium, chloride, and water. It inhibits the pituitary-adrenal axis and, in general, produces effects similar to some adrenocortical steroids.

L. H. Louis and J. W. Conn. *J. Lab. & Clin. Med.* 44: 894, 1954.

Reviews of Recent Books

Advances in Food Research, Volume V, edited by E. M. Mrak and G. F. Stewart, Academic Press, Inc., New York, 1954, pp. 538, $11.50.

This is the fifth volume in the annual series of monographs presenting authoritative reviews on researches in food technology. Volume V deals with such diversified subjects as Oxidative Rancidity and Discoloration in Meat; The Chemistry of the Sugar-Sulfite Reaction and Its Relationship to Food Problems; The Chemistry and Technology of the Pretreatment and Preservation of Fruit and Vegetable Products with Sulfur Dioxide and Sulfites; Flavonoid Compounds in Foods; and the Color Problem in Foods.

A distinguishing feature of this volume is a chapter on Statistical Methods, which should prove of inestimable value to all investigators in food technology, as well as to those in experimental and clinical nutrition. Another chapter treated in detail, which is the first of a series to follow, is in the field of viticulture and enology, entitled Composition of Wines. I. Organic Constituents. The chapter on Rancidity in Meats treats not only of factors causing, but also of methods of preventing rancidity, such as the use of various antioxidants. The chapter on the Chemistry and Technology of the Pretreatment and Preservation of Fruits and Vegetable Products should be of particular interest to the fresh fruit and vegetable industries. In the chapter on the Color Problem of Foods, the mechanism of the fermentation of cocoa is described as a non-oxidative process, previously thought to be due to the action of polyphenoloxidase, but which is mainly accomplished by the destruction of the anthocyanins. Oxidation only takes place during the subsequent sun-drying of the beans. The changes during fermentation are vitally important in determining the flavor and aroma of the final product.—B. SURE

Síndrome Policarencial Infantil (Kwashiorkor) and its Prevention in Central America by M. Autret and and M. Behar, Food and Agriculture Organization of the United Nations, Rome, 1954, p. 74.

In 1951 a joint FAO-WHO committee recommended that a nutrition mission be sent to Central America to study the problem of kwashiorkor and related conditions. This short but thorough report by two experts is the result of the survey.

The syndrome, presumably due fundamentally to protein deficiency in children, has also been called "infantile pellagra," malignant malnutrition, kwashiorkor, etc. For various reasons as stated, the term *infantile pluricarencial syndrome* is recommended.

There is a detailed description of the clinical features, which is enhanced by 11 figures, of which 4 are in color. The skin changes, the edema, and the unhappy facial expressions are striking.

The chapter on treatment emphasizes the importance of protein repletion; skim milk and animal protein are the mainstays, while fat is reduced. Although the vitamin intake must be supplemented, excessive amounts do no good and may even be harmful. The authors stress that the old idea that the syndrome was a hypovitaminosis is erroneous. Basically, the problem is that of general undernutrition and malnutrition, superimposed on a protein deficiency. Lipotropic agents, iron, transfusions, and parenteral fluids are also discussed.

The incidence is certainly higher than can be calculated from cases under medical care. Apparently in many Central American areas it is a serious public health problem. Poverty and ignorance are the chief provocative agents. A consideration of the influence of diet on the syndrome and advice on prevention conclude the report.

With 108 references, 17 tables, and 8 charts, this clear, well-written report is a model in nutritional surveys. S.O.W.

Fat Metabolism. A Symposium on the Clinical and Biochemical Aspects of Fat Utilization in Health and Disease, edited by V. A. Najjar, Johns Hopkins Press, Baltimore, 1954, pp. 185, $4.50.

This little book contains a surprising amount of new and important information on biochemical and clinical advances as related to fat metabolism. It consists of eleven short presentations, all by eminent workers in this active field. Among the subjects treated are certain aspects of obesity, hyperlipemia, coenzyme A, phospholipids, and so on.

As the editor states in his introduction, the book is "mainly aimed at the clinical investigator. A special effort was made to express biochemical events in simple terms. . . ."

The illustrations, references, and index add considerable value to this book, which, in the reviewer's

opinion, is probably the best available exposition of our newer knowledge of lipid metabolism as related to clinical medicine. S.O.W.

Clinical Approach to Jaundice by Leon Schiff, Charles C Thomas, Springfield, Ill. 1954, pp. 113, $3.75.

This monograph of 113 pages gives a systematic and complete summary in brief form of the practical aspects of the study of jaundiced patients. It is emphasized throughout that, despite better modern understanding of the pathologic physiology of jaundice, and despite improved laboratory aids, it is still necessary in all but the most typical syndromes that the physician utilize all his knowledge, judgment, and intuition. Adequate consideration is given to many important aspects of the history and the many intriguing physical findings which may be associated with the jaundice. All of the most useful laboratory tests are reviewed and their place in the study of the patient are evaluated. There is an especially fine section on the use of x-ray studies, and a good review of the help that frequently may be obtained by needle biopsy of the liver. A valuable summary and critical evaluation is supplied under the heading "Limitations and Pitfalls." There are a good bibliography and index. This little volume is highly recommended to practitioners and students.

C. M. MacBryde

Roberts' Nutritional Work with Children by Ethel Austin Martin, University of Chicago Press, 1954, pp. 527, $7.50.

This is the second revision of the original publication in 1927 by Dr. Lydia J. Roberts. The revision, which is a complete rewriting of the previous editions, includes information which is useful as reference material in courses which embody the field of nutrition. It also contains information which is useful to practitioners of medicine who are especially interested in this problem in children.

It is obvious that the author is thoroughly familiar with past and current literature. The help of medical experts has contributed to the accuracy of the information and has assured proper interpretation.

Certain chapters deserve special mention. Chapter II accurately but briefly discusses the role of vitamins in nutrition and also correlates various states of nutrition with certain blood constituents such as proteins, hemoglobin, calcium, etc. Chapter III, which includes a historical review of the problem of growth and development, describes a variety of conditions which influence them. Chapter III gives more information than the majority of pediatric textbooks concerning the use of a variety of charts and guides now in use in evaluating growth and development. Chapter VI gives considerable information on the relationship of nutrition to dental problems in children and is recommended as a useful source of information to pediatricians. Chapter VIII is recommended to those who are interested in the organization and interrelations of various nutrition groups at a local, state, national, and international level. Chapter XIV is especially useful for those concerned with the establishment and operation of school lunch programs; consideration is given to factors and needs which vary from one community or locality to another.

The material of this book is authoritative and is presented in a readable and interesting style. It is recommended to pediatricians as well as nutritionists.

James N. Etteldorf

Textbook of Biochemistry (second edition) by E. S. West and W. R. Todd, The Macmillan Company, New York, 1955, pp. 1313, $12.00.

The need for a second edition in four years bespeaks the popularity of this well-accepted textbook, as well as the recent rapid advances in metabolism, nutrition, and biochemistry. The authors, from the Department of Biochemistry, University of Oregon Medical School, have encompassed an amazing amount of material in this book, but the excellent index and numerous figures, tables, and charts prevent the information from being hidden from the reader who consults it for reference.

Among the subjects of recent interest which are covered are alpha-lipoic acid, the pancreatic hyperglycemic factor (which should be called "glucagon"), coenzyme A, and various antimetabolites.

Nutrition is particularly well covered, and although the arrangement of chapters occasionally leads to a divided discussion and some duplication (e.g. cocarboxylase, porphyrins) nevertheless essentially everything of importance is included.

This textbook can be safely recommended as a solid, substantial, up-to-date reference work for student and advanced worker. S.O.W.

Eating Together by Camille Macauley, Farrar, Straus and Co., New York, 1950, pp. 419, $3.95.

This book is written by a homemaker who was faced with the problem of meal planning and preparation for a family of five which included a diabetic adult and a diabetic child. In the first section of the book the diabetic patient and members of his family are led to an understanding of the essential nature of diabetes and the important principles in its treatment. The author describes in a very readable style some of the fears and problems which confront the diabetic individual, and gives practical suggestions for meeting them.

The second part of the book contains more than 100 menus for breakfast, at-home luncheons, lunch-

box meals, and dinners. All of the menus are planned around a diet prescription of protein, 90 Gm.; fat, 100 Gm.; and carbohydrate, 200 Gm. These menus can be readily adjusted to other prescriptions by modifying the amounts of meat, fat, or bread. This book was published prior to the adoption of the exchange lists now so widely used, and hence computations are more detailed than they need be. It would be necessary for the patient to have a good understanding of the calculation of his own diet and the method of substitutions to be used in the listed menus. In view of the wide variations in food composition, one might also question the need for weighing the foods.

The menus which are given illustrate how meals for the family may also become meals for the diabetic; and that food for the diabetic need not be monotonous. The protein, fat, and carbohydrate calculations for the stated portions are given for each food item. Family-size recipes for food combinations, together with the calculated values for one portion, are also given with each menu.

The book includes special sections on between-meal feedings, dining in a restaurant, fountain lunches, special occasion menus, tables of food substitutions, and a table of food values. The patient who is facing the problems of a monotonous diet and who has the time to make the necessary adjustment of the menus may find this book helpful. The information appears to be accurately stated. C.R.

Food Preparation Recipes by Kathryn Bele Niles, John Wiley & Sons, Inc., New York (Chapman & Hill, Ltd., London), 1955, pp. 372, $3.90.

In addition to its large repertoire of recipes, this volume attempts to inculcate the principles underlying their formulation. To this end, the introductions to the various sections discuss the "basic methods" applicable to a given type of food (eight for eggs, six for meats, and so on), and "master recipes" are followed by suggestions for substitutions and variations. This makes for an analytic, rather than a rote approach to culinary operations; it should, in time, render the attentive student relatively independent of the teacher, and able to work out her own variations of the themes presented. The inexperienced will particularly appreciate the criteria listed under the heading "Standard"—which describe in some detail the qualities (texture, color, consistency, etc.) the final product ought to possess, thus answering the tyro's frequent question: "How do I know when . . .?"

What to do if a custard curdles, how variety meats can supply high quality protein cheaply, and why the omelette skillet should never be washed or polluted with other foods—all these matters and more are helpfully expounded by the Home Economics Director of the Poultry and Egg National Board.

The volume is attractively designed and spiral-bound to open flat. C.-J. H.

Books received for review by the *American Journal of Clinical Nutrition* are acknowledged in this column. As far as practicable, those of special interest are selected, as space permits, for a more extensive review.

A Symposium on Amino Acid Metabolism, edited by W. D. McElroy and B. Glass, The Johns Hopkins Press, Baltimore, 1955, pp. 1048, $12.50.

Nutritional Data (second edition), by H. A. Wooster, Jr., H. J. Heinz Co., Pittsburgh, 1954, pp. 155.

Kwashiorkor, by H. C. Trowell, J. N. P. Davies, and R. F. A. Dean, Edward Arnold (Publishers) Ltd., London (The Williams & Wilkins Co., Baltimore), 1954, pp. 308, $10.00.

Abstracts of Current Literature

ABSTRACTERS

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NUTRITION AND THE LIVER

The importance of protein in the treatment of chronic liver disease has been recognized as a life-saving dietary measure since the report of Patek and Post. However, the lack of tolerance of the patient to protein feeding may force a compromise with respect to the allowances of these substances in the diet. It has been shown that the ammonia arising from deamination of amino acids may not be adequately disposed of in patients with liver disease, giving rise to mental symptoms identified as hepatic coma. A reduction of dietary protein may effect a striking improvement in this ominous condition.

Dietary Protein in the Genesis of Hepatic Coma. R. Schwartz, G. B. Phillips, J. E. Seegmiller, G. T. Gabuzda, Jr., and C. S. Davidson. *New England J. Med.* 251: 685, 1954.

Three patients were studied who had chronic alcoholism, cirrhosis of the liver, and neurologic complications, and showed an intolerance to protein when the dietary protein exceeded certain levels. This intolerance was shown by a characteristic "flapping" tremor which preceded coma.

The first patient, a man with chronic alcoholism and cirrhosis of the liver, showed the "flapping" tremor on a daily dietary intake of 85 Gm. of protein; it diminished and finally disappeared when the protein level was decreased to 50 Gm. daily. Three times the daily dietary protein was increased to 75 Gm. daily. Each time tremor appeared, disappearing when the daily dietary protein was decreased to 50 Gm. Electroencephalograms taken during the period when the "flapping" tremors appeared were characteristic of impending coma.

The other two cases were complicated by hyponatremia. The serum sodium concentration may have some effect on the mental symptoms, though it was thought that this was not of primary etiologic significance. These two patients, like the first one, showed improvement when the dietary protein level was decreased.

The possible mechanisms of the protein intolerance are discussed. A relation to ammonia metabolism seems to exist. Severe hepatic damage may result in an inability of the liver to dispose of excess ammonia. Another possibility is that the extensive portal collateral circulation would cause portal blood containing high levels of ammonia to bypass the liver, resulting in a general elevation of blood "ammonia," to which the brain is sensitive.

It is well established that a nutritious diet is necessary in cases of liver cirrhosis. This paper only emphasizes the need for protein restriction at the onset or presence of impending hepatic coma. The individual tolerance of the patient should determine the level of daily protein intake.—M. W. BATES

Previous unsupported clinical reports had emphasized the improvement in hepatic cirrhosis following administration of choline and methionine. Such therapy is still recommended in some quarters. However, it has been shown that the usual high protein diet prescription provides more than adequate amounts of these substances. The following investigations are an important reappraisal of the problem of the lipotropic action of protein and certain amino acids.

On the Lipotropic Action of Protein. A. E. Harper, D. A. Benton, M. E. Winje, and C. A. Elvehjem. *J. Biol. Chem.* 209: 171, 1954.

Investigations in this field have centered largely on whether the lipotropic activity of a protein can be attributed entirely to its content of cystine and methionine. More recently it has become apparent that protein and the amino acids, threonine and glycine, were effective in reducing the deposition of fat in the liver of young rats fed low protein diets containing choline nor methionine, in amounts sufficient to meet examined as lipotropic actions of proteins, rather than that of the methionine contained in them. Neither choline nor methionine, in amounts sufficient to meet the stated requirements of the rat for these compounds for growth and for the prevention of fatty infiltration, prevented some excess of fat from accumulating in the liver. Only when either the protein or the threonine content of the diet was increased was the fat content of the liver reduced to what is considered the normal range. The results obtained by adding methionine and choline to casein diets which varied from 9 to 18 per cent, with and without supplements of gelatin and DL-threonine, were not easy to interpret. It is suggested that there are two distinct effects of dietary protein on fat deposition in the liver; first, the effect of methionine, in sparing choline by providing methyl groups for choline synthesis, and secondly, the effects of other amino acids, partial deficiencies or imbalances of which cause fat to accumulate in the liver.

It appears that the secondary effect of protein in reducing the deposition of fat in the liver is a reflection of the amino acid-deficient diets used in most studies. Threonine and glycine are required to insure minimal fat deposition in the liver when low casein diets are fed. With other proteins, other amino acids may be required. The authors call attention to unpublished results which indicate that glycine is more effective than threonine when albumin diets are fed.—M. K. HORWITT

The cause of ascites in liver disease lies principally in the increased portal pressure associated with the cirrhotic process. However, fluid retention in this condition can be effectively combatted in many instances by drastic sodium restriction.

Control of Ascites in Hepatic Cirrhosis. M. Atkinson, A. Paton, and S. Sherlock. *Lancet* 1: 128, 1954.

Ascites due to hepatic cirrhosis can be controlled by restricting the sodium intake to 22 milliequivalents daily. This relieves the necessity of repeated paracentesis, which by removing large quantities of protein from the body in the ascitic fluid is particularly dangerous when the ability to synthesize albumin is reduced by liver disease.

Four cases, illustrating the treatment, are presented in some detail. By the use of sodium-free protein supplements, 120 Gm. of protein could be given daily in a diet providing 1500 to 2000 calories. Mercurial diuretics and ammonium chloride were also given. Serum sodium and potassium were estimated weekly.

A hospital stay of two or three months is usually necessary before treatment can be continued on less rigorous lines as an outpatient.—F. E. HYTTEN

The liver is the principal site of vitamin A formation from the carotenes and represents the chief repository of this vitamin.

Vitamin A and Carotenoid Levels in Blood Serum with Special Reference to Values Observed in Diseases of the Liver. E.-M. L. Ditlefsen and K. F. Stoa. *Scandinav. J. Clin. & Lab. Investigation* 6: 210, 1954.

The vitamin A level in 100 normal subjects varied from 15 to 45 μg. per 100 ml., and the normal carotenoid level varied from 50 to 200 μg. per 100 ml.

Patients with chronic renal disease and anemia showed a considerable increase in vitamin A levels, while slightly increased values were found in alcoholics.

Obstructive jaundice was associated with a decrease in carotenoid level without a significant decrease of the vitamin A content. Hepatocellular damage was associated with the reverse, that is, normal carotene but depressed vitamin A levels.—S. O. WAIFE

Elsewhere in these abstracts vitamin B_{12} has been recommended in the treatment of infectious hepatitis. One of the actions attributed to vitamin B_{12} in the livers of experimental animals is that of increasing nucleic acid synthesis, and increasing liver cell regeneration, as in the following study.

Liver Changes in Vitamin B_{12} and Riboflavin-Deficient Rats Before and After Partial Hepatectomy. B. S. Schweigert, H. E. Sheid, and M. Downing. *Am. J. Physiol.* 178: 338, 1954.

This study investigated the role of vitamin B_{12} in nucleic acid synthesis. Vitamin B_{12}-deficient rats had a higher mortality and showed less liver regeneration than vitamin B_{12}-supplemented rats, riboflavin-deficient rats, or control animals. Desoxyribonucleic acid and ribonucleic acid were reduced in vitamin B_{12} deficiency but not in riboflavin deficiency. These studies are evidence for the specific role of vitamin B_{12} in nucleic acid synthesis.—M. J. OPPENHEIMER

The type of circulatory impairment affecting the hepatic cells in liver disease is one in which the internal hemodynamics are altered by fibrosis and congestion within the liver lobules. Devascularization of the liver may produce a somewhat analogous type of anoxic change affecting hepatocellular function.

Liver Function Following Partial Devascularization of the Liver. H. L. Popper, N. C. Jefferson, E. Wulkan, and H. Necheles. *Am. J. Physiol.* **178**: 321, 1954.

Following partial devascularization experiments there was interference with the blood supply of the liver. Despite this, there were no important changes in serum cholesterol, cholesterol esters, or blood sugar. The degree of devascularization and the time elapsed were not factors. Elevation in serum alkaline phosphatase was not proportional to the degree of devascularization. Serum albumin was markedly reduced and the albumin/globulin ratio was reversed. These last results are proportional to the reduction in hepatic circulation, and here the duration of devascularization was a factor. There was no tendency to bleeding.—M. J. OPPENHEIMER

In liver disease, carbohydrate intolerance has long been recognized as a commonly encountered condition. The following report indicates that the liver plays a role in the peripheral utilization of glucose.

Influence of the Liver on Uptake of Glucose by Extrahepatic Tissues. S. Lang, M. S. Goldstein, and R. Levine. *Am. J. Physiol.* **177**: 447, 1954.

The hind legs of eviscerated dogs have a peak utilization of glucose of 600 mg./Kg./hr. In the intact animal they can use as much as 1500 mg./Kg./hr. Excessive amounts of insulin do not restore the difference in carbohydrate use in the eviscerated dog. In the insulin-free depancreatized animal high blood levels alone can produce an increase in glucose uptake. The authors are of the opinion that glucose uptake in the peripheral tissues of the leg depends upon the liver in the presence of a carbohydrate load. Cross transfusion between an eviscerate and intact dog can demonstrate this. The liver promotes peripheral storage and transformation of excess carbohydrate. The liver is not a major depot itself.—M. J. OPPENHEIMER

CALCIUM METABOLISM

The absorption and retention of calcium is under the direct influence of vitamin D; however, the presence of oxalates and phytins in the intestinal tract reduces the absorption of calcium through the formation of insoluble salts. In addition, the ratio of calcium to phosphorus in the diet was formerly believed to be significant in the retention of these substances. The following paper confirms other observations that the retention of calcium is proportional to the level of intake rather than dependent upon the phosphorus ratio or other sources of calcium.

The Relation of Calcium-to-Phosphorus Ratio to the Utilization of these Minerals by 18 Young College Women. M. B. Patton and E. D. Wilson. *J. Nutrition* **50**: 373, 1953.

The ratio of calcium to phosphorus and the amount of vitamin D in the diet have been shown to be factors in the development and cure of rickets in both experimental animals and young children. Based upon observations among several species, Sherman recommends a calcium-to-phosphorus ratio somewhere between one and two during rapid growth and calcification. Macy, however, has stated that insufficient data are available to show the significance of this ratio in food in relation to the retention of calcium during growth. Patton and Wilson, therefore, considered it of interest to investigate further the significance of the calcium-to-phosphorus ratio to the utilization of these minerals in 18 normal healthy young college women.

A basal diet, furnishing 344 mg. of calcium and 766 mg. of phosphorus, was supplemented by varying amounts of calcium or phosphorus, or both, to give ratios of calcium to phosphorus varying from 1:0.50 to 1:3.97. The calcium intake levels were 344, 944, and 1544 mg.; the phosphorus levels, 766, 1066, and 1366 mg. Sodium glycerophosphate, calcium carbonate, sodium phosphate, and calcium phosphate were used as supplements to the basal diet. At each level of calcium intake, an increase of phosphorus intake was without significant effect on calcium retention. However, when phosphorus was kept constant, an increase in calcium intake resulted in significant increase in calcium balance. With constant phosphorus intake, phosphorus balances were not affected by an increase in calcium intake. An increase in phosphorus intake from the amount in the basal diet, 766 mg. to 1366 mg., resulted in a higher mean phosphorus balance only if the calcium level was as much as 944 mg. The indications were that the balances of both minerals were more closely related to intakes than to the ratios of calcium to phosphorus.—B. SURE

The formation of insoluble calcium complexes in the intestinal tract results from the feeding of spinach (oxalate) or chelating agents. It would appear from the following study that calcium phosphate salts permit only a limited exchange of calcium across the mucosa.

Alterations in Dynamics of Calcium Metabolism by Intraintestinal Calcium Reservoirs. R. O. Thomas, T. A. Litovitz, and C. F. Geschickter. *Am. J. Physiol.* **176**: 381, 1954.

Continuous oral intake of spinach (for 7 or 18 days) increases the rate of disappearance of calcium from the blood. The fecal excretion rate is also increased. After this diet, the uptake of radiocalcium by bone is depressed, and the urinary excretion is less. When sodium versene or calcium dibasic phosphate is given by stomach tube, similar effects on blood disappearance and fecal excretion are produced, but bone up-

take and urinary excretion are not changed by single doses of these substances. Oxalic acid of spinach and sodium versene form complexes of nonionizable calcium, which are relatively insoluble and act by virtue of establishing a nonabsorbable calcium reservoir in the intestinal tract. This reservoir competes for blood calcium by exchange across the intestinal mucosa. This in turn lowers bone uptake and urinary excretion. The blood calcium reaches a peak 20 minutes after radiocalcium chloride is introduced into the lumen of the duodenum. After this, the disappearance rate from blood resembles that of intravenous radiocalcium. The maximal concentration in blood by intravenous injection is never reached by the duodenal route, although blood values stay higher.—M. J. OPPENHEIMER

The excretion of calcium is increased and the exchange of this nutrient into bone salts is decreased in aging animals. This may be an important clue in the study of osteoporosis. The reduction of the calcium intake noted in several series of patients with this condition may, in the light of the above data, serve to explain some of the findings in osteoporosis.

Effect of Age Upon the Physiological Behavior of Calcium in Cattle. S. L. Hansard, C. L. Comar, and G. K. Davis. *Am. J. Physiol.* 177: 383, 1954.

Labeled calcium was used in these investigations. Excretion rates of unabsorbed calcium were increased threefold in old animals. Exchange of ionized calcium between blood and other compartments showed a significant decrease with age. The relative percentage of exchangeable calcium between bone and soft tissue was also decreased as age increased.—M. J. OPPENHEIMER

The Effect of Diet on the Metabolic Alterations of Paraplegia. D. M. Wyse and C. J. Pattee. *Canad. M. A. J.* 71: 235, 1954.

Among patients with paraplegia there is a danger that disuse osteoporosis will lead to high urinary levels of calcium and to the development of urinary calculi. To see whether alterations in calcium and phosphorus intake could modify this factor, the authors studied two paraplegics for 3 to 6 days.

It was found that when hypercalcuria was present, a low calcium intake (0.3 Gm.) did not reduce the calcium excretion. On the other hand, a high calcium intake (2.5 Gm.) increased the calcuria only if the patient was already in a positive calcium balance. A high Ca intake increased the likelihood of maintaining a positive calcium balance without significantly altering urinary calcium excretion.

A low phosphorus intake was associated with a reduction in urinary phosphorus. Since two-thirds of urinary calculi in paraplegics are of the phosphate variety, it would seem that a diet both high in calcium and low in phosphorus would be the intake of choice in patients who are immobilized. This would involve severe milk restriction and the administration of a calcium salt such as the lactate.—S. O. WAIFE

The converse of vitamin D-resistant rickets may be the following condition of hypercalcemic syndrome in infancy. An increased sensitivity to vitamin D should be explored as the likely explanation for this interesting condition.

Idiopathic Hypercalcaemia in Infants with Failure to Thrive. R. D. G. Creery, and D. W. Neill. *Lancet* 2: 110, 1954.

A detailed description of this syndrome in 16 infants is presented. Twelve of the cases began at or before six months of age—one at birth—but they were generally not recognized for some months. Thirteen were given no specific treatment apart from reducing the vitamin D intake, although in several a low calcium diet was also given. One infant received cortisone and showed a striking clinical and biochemical improvement, but it is possible that this case was already recovering before treatment. Recovery took from one to 22 months.

It is regarded as important that none of the infants was breast fed and it is claimed that "the only constant etiological factor is a high calcium intake from early infancy, usually associated with a more than adequate amount of vitamin D in the diet," but this can scarcely apply to the case where the condition is said to have begun at birth.

A possible mechanism for the production of the syndrome is discussed.—F. E. HYTTEN

The Idiopathic Hypercalcaemic Syndromes of Infancy. K. G. Lowe, J. L. Henderson, W. W. Park, and D. A. McGreal. *Lancet* 2: 102, 1954.

Six "mild" cases and one "severe" case of this condition are described in considerable clinical and biochemical detail.

The "severe" case, whose condition did not improve during 18 months of treatment and who subsequently died, was characterized by physical and mental retardation, hypertension, albuminuria, and otosclerosis. Histological examination of the kidney revealed extensive damage but shed no light on the etiology.

The six mild cases (five of them in the first six months of life) presented with vomiting, constipation, and failure to gain weight. There was a somewhat raised blood urea, and a blood calcium level which varied between 11.6 and 15.6 mg. per 100 ml. when the cases were first examined. One of these children died of intercurrent disease, but the other five all showed clinical recovery, even though the hypercalcemia itself tended to persist.

It is suggested that the "mild" and "severe" syndromes may be variants of the same disease process,

but there is no real evidence to support this.

The most likely etiological factor is thought to be hypervitaminosis D (or a hypersensitivity to vitamin D) and it is suggested that excessive administration of alkaline medicines may be an aggravating factor. —F. E. HYTTEN

Increasing evidence is accumulating to indicate that the principal function of the parathyroid hormone is the regulation of the solubility of the calcium bone salts. The phosphaturia observed after parathormone administration may be another direct effect or may be the result of changes in the renal excretion rates of calcium and phosphates produced by changes in their blood levels.

The Parathyroidal Function in Long-Term Dietary Experiments. B. Engfeldt, S. O. Hjertquist, and J. R. E. Strandh. *Acta Endocrinologica* 15: 119, 1954.

Since the blood levels of calcium and phosphorus seem to regulate parathyroid activity, these authors studied the effect of long-term experiments (up to one year) on rats with diets containing varying amounts of calcium and phosphorus.

On a diet rich in phosphorus (instead of the 1:1 ratio among control animals, the calcium to phosphorus ratio was 1:16), there was a continuous hyperfunction and hyperplasia of the parathyroid glands. The blood phosphorus values fell soon after the diet was instituted and remained at a low level. Calcium values were normal or only slightly reduced. On the other hand, on the high calcium diets (8:1 ratio), atrophy of the parathyroids was observed. The blood calcium rose early in the experiment but then fell to subnormal levels. On the other hand, the blood phosphorus levels rose in the latter part of the experiment.

In this study, the existence of a direct correlation between the calcium and phosphorus in the diet and the concentration of these substances in the blood could not be found. Neither was there a clear inverse relationship between calcium and phosphorus in the blood, as has been pointed out by others. As a result of the analysis of the data, the authors feel that the blood calcium or a fraction of it may be the primary factor in the regulation of parathyroidal function. —S. O. WAIFE

The use of calcium infusions to alter the level of parathormone production, as described by Howard, may prove a useful tool in the diagnosis and study of certain metabolic bone diseases. However, more information concerning the influence of altered blood levels of calcium and phosphorus upon the renal responses and excretion rates is necessary.

The Metabolic Effects of Intravenous Administration of Calcium. L. H. Kyle, M. Schaaf, and L. A. Erdman. *J. Lab. & Clin. Med.* 43: 123, 1954.

Control and experimental subjects with and without metabolic bone disease were given comparable intravenous infusions of calcium gluconate in isotonic saline solution. An equilibrium state was assumed following a preliminary period of at least three to five days, during which a calculated diet presumed to contain constant quantities of calcium and of phosphorus was prescribed. Five-hundred cc. of infusion fluid were given over a three-hour period so that about 10 mg. of calcium per kilogram of body weight were administered. Ambulatory status in controls and in patients was dissimilar. The serum inorganic phosphorus concentration rose, in association with the increase in serum calcium, in control subjects and in a small number of patients with osteomalacia and osteoporosis. This was not found in isolated examples of other disorders and quite clearly contradicts classical notions of a reciprocal relation between concentrations of serum calcium and of serum inorganic phosphorus. Nevertheless, this lack of reciprocity must be assumed to be genuine in view of previous corroborative evidence in man and in animals. Since the urinary excretion of phosphate did not appear to fall during the calcium infusion, it is postulated that the increased serum inorganic phosphorus concentration is attributable to movement of phosphate from intracellular to extracellular fluid as a consequence of temporary depression of parathyroid activity. Supposed differences in renal response to calcium in controls as against experimental subjects are presented and theoretical implications outlined in conjunction with possible application to problems of differential diagnosis. It would appear, however, that much of the relevant data do not lend themselves to rigorous analysis. A similar problem is posed by the interpretation of presumed differences in magnitude of calcium retention.—R. TARAIL

LIPODYNAMICS

The formation of fat within adipose tissue is a matter of considerable interest. There is evidence that fat is formed from acetate derived from carbohydrate substrate; glycogen has been demonstrated within adipose tissue and may represent a source of fat. In addition, fatty acids and their esters also represent an important source of fat deposited in the tissues. The transport of these substances into adipose tissue is the subject of the following reports. The origin of the fatty acids may be dietary or from endogenous synthesis.

The Transport of Lipids into Adipose Tissue. I. Stern and B. Shapiro. *Metabolism* 3: 539, 1954.

Rats starved for five days provided the fat-depleted adipose tissue for an investigation as to the vehicle by which fatty acids are carried into the tissue and the type of enzyme activity involved in the transport. When adipose tissue was incubated in serum, there was no decrease observed in the phos-

pholipid and cholesterol ester fractions. Apparently, tissue is capable of absorbing neutral fatty acid compounds, for fatty acids disappearing from the medium could be recovered in good yield from the tissues. The absorption of fatty acid esters was inhibited by sodium fluoride; however, the free (non-esterified) fats were absorbed in the presence of this inhibitor. This suggests that the transport of fatty acids into the cells involves an enzymatic reaction acting on the ester linkage.—C. R. SHUMAN

Disposition of Biosynthesized Fatty Acids in Normal and Fasted Rats. J. G. Coniglio, C. E. Anderson, and C. S. Robinson. *Am. J. Physiol.* 177: 69, 1954.

Rats, either fed or after a three-day fast, showed no difference in the distribution of biosynthesized fatty acids between neutral fat and phospholipids in various tissues. The ratio of these acids in neutral fat to that in phospholipid of liver was close to 1.0. However, in the intestine phospholipid fatty acids were more heavily labeled. Ratios of intramuscular and subcutaneous fat were different. These ratios were similar for saturated and unsaturated hepatic fatty acids whether in fed or fasted animals. The saturated acids were more heavily labeled in carcass fat of fasted animals. There was no difference in total activity in excreted fecal fatty acids for fed or fasted rats. In starved animals the specific activity of fatty acids was higher.—M. J. OPPENHEIMER

Function of the Reticuloendothelial System in Removal of Emulsified Fat from Blood. W. R. Waddell, R. P. Geyer, E. Clarke, and F. J. Stare. *Am. J. Physiol.* 177: 90, 1954.

Fat emulsions were dyed with Sudan Black and Sudan IV. These were injected into rats and the livers and spleens examined to determine where the fats were localized. The reticuloendothelial system was "blocked" and fat was no longer deposited in those cells. Clearance rates of emulsified fats from the blood stream were not significantly changed. When reticuloendothelial cells were blocked or laden with fat the emulsified fat particles entered polygonal cells of liver and splenic pulp cells.—M. J. OPPENHEIMER

Fats, like certain electrolytes and other substances, are excreted through the intestinal mucosa, according to the following report.

Fecal Fat on an Essentially Fat-Free Diet. G. T. Lewis and H. C. Partin. *J. Lab. & Clin. Med.* 44: 91, 1954.

Three hospitalized patients, two of whom had recognized gastrointestinal disorders, were studied while on a negligible fat intake. The diet was evidently synthetic, at least in part, and was low in content of certain minerals and perhaps vitamins as well. Fecal fat reportedly averaged about 2 grams per day. In one subject, this appeared to be clearly established by intermittent measurements over an interval of about two months; in a second patient, sporadic observations lasted at least six days; in a third, no definite interval could be inferred. The data were interpreted as consistent with the theory that fecal lipid is excreted through the intestinal mucosa.—R. TARAIL

There has been some controversy concerning the effect of vegetable fats upon serum lipids. In the following study, the high vegetable fat diets were found to produce a decrease in serum lipids. Further observations are necessary for clarification of these changes and of the possible mechanisms involved.

Effects of High-Fat Diets on Serum Lipids. Animal vs. Vegetable Fats. L. W. Kinsell. *J. Am. Dietet. A.* 30: 685, 1954.

The author reports that "the administration of formula or mixed diets containing large amounts of vegetable and no animal fat to human subjects with a wide variety of diseases resulted in a major decrease in the level of certain of the serum lipid components. The precise explanation for these changes is still to be obtained."

This paper shows graphically detailed data for four subjects with rheumatoid arthritis, myxedema, nephrosis, or splenomegaly. The first two patients were given high vegetable fat formula diets for 40–65 days, and the plasma or serum cholesterol (free and ester forms) and phospholipids were plotted at frequent intervals. Hormones were given to the arthritic patient but not to the other. The patient with nephrosis received predominantly the vegetable fat formula diet, though it appears that there may have been 3 to 4 days of high animal fat. Hormonal medication was reported during the last 25 days of the 90-day study. The patient with splenomegaly was under observation for 135 days, during which ACTH or stigmasterol was administered for approximately 40 days. The diet of this subject supplied approximately 4000 calories, with 1000–1500 calories from carbohydrates, 300–700 calories from protein, and the remainder from fat, either vegetable or animal.

In most of the data, free cholesterol of the blood changed less than did the other constituents. It is difficult to evaluate these data due to the method of plotting and to the lack of detail concerning the diets. No information is given concerning the foods used in the mixed diets or the vitamin and mineral supplements used with the formula diets.—J. M. SMITH

The fatty degeneration of the liver produced by ethionine is apparently related to its antilipotropic action as a methionine antimetabolite. The ability

of epinephrine to promote fat mobilization is demonstrated.

Reduction of Serum Lipids and Lipoproteins by Ethionine Feeding in the Dog. H. Feinberg, L. Rubin, R. Hill, C. Enterman, and I. L. Chaikoff. *Science* 120: 317, 1954.

Ethionine, the ethyl analogue of methionine, can induce fatty livers in rats. When dogs are fed 125 mg. of *dl*-ethionine daily there is a prompt reduction in the level of serum fatty acids, phospholipids, and cholesterol. (The serum cholesterol fell from 180 to 20 mg. per 100 ml. serum.)

Ethionine also caused a marked reduction in the level of low- and high-density lipoproteins. Removal of ethionine from the diet resulted in a prompt restoration of all lipid and lipoprotein fractions to normal levels.—S. O. WAIFE

Role of Epinephrine in the Physiology of Fat Mobilization. I. G. Wool, M. S. Goldstein, E. R. Ramey, and R. Levine. *Am. J. Physiol.* 178: 427, 1954.

When female rats are fasted and then fed ethionine, they develop fatty livers. This result is prevented by prior adrenalectomy. Large doses of cortisone do not correct this. However, when small amounts of cortisone are combined with epinephrine, the ability to mobilize fat is restored. Fat mobilization was also reduced by ergotamine and adrenal demedullation to levels resembling those in total adrenalectomized rats. The ability to develop a fatty liver in the demedullated rats was restored by epinephrine. The autonomic nervous system and the closely related adrenal medulla have important roles in fat metabolism.—M. J. OPPENHEIMER

High fat feedings have been shown by certain workers to increase blood coagulation. Several conflicting reports have appeared which contradict this finding. It has been suggested that re-used syringes are responsible for the increased rate of coagulation of blood, rather than the effect of fat upon the clotting mechanism. The following reports deal with the effect of fat upon blood clotting and viscosity. Among the clinical implications is the matter that in multiple sclerosis, a low fat diet has been advised because of the hypothesis that the changes described below may enhance or be responsible for the demyelinating process occurring in this disease.

Lack of Effect of Fat Ingestion on Blood Coagulation. P. R. Manning and R. L. Walford. *Am. J. Med. Sc.* 228: 652, 1954.

The influence of the ingestion of fat as cream or a butter-egg-bacon feeding upon the coagulation time was observed in 18 patients. In addition, the clot retraction and prothrombin consumption were determined. The ingestion of cream was followed by lipemia, the degree of which was correlated with the fat content of the cream samples. The oral ingestion of varying amounts and types of fat had no significant effect upon blood coagulation. The age of the patients did not influence the results. There was no effect observed on clot retraction or on prothrombin consumption times.—C. R. SHUMAN

Effect of High Fat Feedings on Viscosity of the Blood. R. L. Swank. *Science* 120: 427, 1954.

Blood viscosity was measured as the time required for 0.1 ml. of blood to flow through a 25-gauge needle inserted in a vein under standardized conditions.

Hamsters on a high fat diet were given a single fat meal of 2 to 3 ml. of 35 per cent cream and the blood viscosity determined at frequent intervals afterwards. There was an increase in viscosity, first noted about 3 hours after the cream feeding, and reaching its peak in 6 to 9 hours. By the end of 24 hours, the viscosity returned to normal, but there was a significant drop in hematocrit. This is thought to be due to "sticking" of erythrocytes. The increase in viscosity was associated with hyperemia, cyanosis, and a reduced bleeding tendency.

Heparin injections produced a transient reduction of the excess viscosity.—S. O. WAIFE

ABSORPTION OF VITAMIN B_{12} AND CLINICAL APPLICATIONS

While efforts directed toward the isolation and identification of intrinsic factor are rapidly reaching fruition, methods for measuring the activity of this agent in humans have been developed in several clinics. Various techniques for determining absorption levels of radioactive vitamin B_{12} have found the widest application and are being utilized to study these problems.

Absorption of Radioactive Vitamin B_{12} after Total Gastrectomy, Relation of Macrocytic Anemia and to the Site of Origin of Castle's Intrinsic Factor. J. A. Halsted, M. Gasster, and E. J. Drenick. *New England J. Med.* 251: 161, 1954.

A controversy exists as to the exact location of intrinsic factor secretion. In swine, the intrinsic factor is secreted primarily in the pyloric end of the stomach, and in man in the fundus of the stomach. Secretion may occur outside of the stomach. An argument in favor of extragastric secretion is that all patients with total gastrectomy do not develop pernicious anemia. If secretion occurred only in the stomach, these patients should develop pernicious anemia.

Since intrinsic factor is needed for the absorption of B_{12}, the patient with pernicious anemia is not absorbing B_{12} due to a lack of intrinsic factor. Oral administration of B_{12} to these patients is ineffective unless given in large amounts, but if the dose is

administered along with a source of intrinsic factor, such as gastric juice, only one microgram of B_{12} is necessary for a measurable hematologic effect.

Measuring the hematologic response has been the only available way of assaying intrinsic factor preparation. Since this factor results in increased absorption of B_{12}, another way of measuring intrinsic factor activity would be to measure amount of cobalt^{60}-labeled vitamin B_{12} left in feces after absorption takes place.

The authors fed cobalt^{60}-labeled vitamin B_{12} to 11 patients with total gastrectomy, 11 control subjects, 7 patients with proved pernicious anemia, and 3 with idiopathic achlorhydria.

On the average, the controls excreted in their feces 33 per cent of the test dose, the patients with idiopathic achlorhydria excreted 35 per cent. Patients with pernicious anemia excreted practically all (93 per cent) of the administered cobalt^{60}-labeled vitamin B_{12}. When a source of intrinsic factor was given to the patients with pernicious anemia, fecal excretion was significantly decreased to 38 per cent of the oral dose.

None of the patients with total gastrectomy had macrocytic anemia, except one who had had pernicious anemia before the operation. Every patient excreted practically all (87 per cent) of the radioactive vitamin B_{12} when it was given without intrinsic factor, but when it was administered with gastric juice the excretion was decreased to 20 per cent. These observations would indicate that a patient with a total gastrectomy does not absorb B_{12} because of a lack of intrinsic factor, and that the main site of secretion of the intrinsic factor in man is in the stomach. Since patients with achlorhydria absorbed vitamin B_{12} in normal amounts, free hydrochloric acid is not necessary for its absorption.

The question arises why pernicious anemia does not always follow total gastrectomy if the stomach is the main source of intrinsic factor. This paradox may be explained by four factors. (1) Not many patients survive the operation long enough for the macrocytic anemia to develop. (2) It is difficult to know exactly how long it takes the anemia to develop after intestinal absorption of B_{12} has stopped. (3) It is difficult completely to remove all stomach tissue. (4) Most patients probably receive multivitamin preparations which would include B_{12} after the removal of the stomach.

This method of measuring the absorption of vitamin B_{12} can be used clinically in the diagnosis of pernicious anemia before hematologic changes develop. It can also be used in assaying intrinsic factor preparation.—M. W. Bates

The method described below has received considerable attention as a means of quantitatively measuring the absorption of vitamin B_{12} and may serve as a means of assaying intrinsic factor activity.

Uptake of Radioactive Vitamin B_{12} by the Liver in Humans: Test for Measurement of Intestinal Absorption of Vitamin B_{12} and Intrinsic Factor Activity. G. B. J. Glass, L. J. Boyd, G. A. Gellin, and L. Stephanson. *Arch. Biochem. & Biophys.* 51: 251, 1954.

Between the sixth and tenth day following the ingestion of a single dose of 1–2 μg. of radioactive Co^{60} vitamin B_{12}, the uptake over skin projections of the liver was measured with a scintillation detector. The rate of disappearance of absorbed vitamin B_{12} from the liver was exceedingly slow, 86 to 94 per cent of the activity being retained after 3 months. This technique provides a quantitative method for the intestinal absorption of vitamin B_{12}.

In patients with pernicious anemia or sprue, no or negligible hepatic uptake of radioactive vitamin B_{12} was observed, the counts being in the neighborhood of zero and indicative of highly defective intestinal absorption of the vitamin in these diseases. When the oral test was repeated with a similar single dose of radioactive vitamin B_{12} to which 75–100 ml. of normal human gastric juice or potent intrinsic factor concentrate from the human or hog stomach was added, the presence or absence of intrinsic factor in the stomach of individuals tested could be determined. If the defect in vitamin B_{12} utilization depends, as in pernicious anemia, on the absence of intrinsic factor from the stomach, it will be corrected by addition of normal gastric juice or intrinsic factor preparation, and a significant increment in the hepatic uptake of radioactive vitamin B_{12} will be obtained.

The authors believe that the technique described will be useful in detecting the presence or absence of intrinsic factor in the stomach of any individual studied.—M. K. Horwitt

The entire problem of the intrinsic factor-vitamin B_{12} interrelationship has been thoroughly presented in the following review, which should be read in its entirety by those interested in this subject. The reader is also referred to the Jan.-Feb. 1955 issue of this Journal.

Recent Studies on Intrinsic Factor and the Utilization of Radioactive Vitamin B_{12}. R. F. Schilling. *Fed. Proc.* 13: 769, 1954.

Studies on the absorption, excretion, and distribution of vitamin B_{12} and of the relationship of the vitamin to the intrinsic factor have been aided by the availability of Co^{60} B_{12}. The fecal excretion of radioactive vitamin B_{12} administered to patients with pernicious anemia is reduced from 72 to 100 per cent to a range of 25 to 60 per cent when intrinsic factor is added. The absorption of vitamin B_{12} bearing the cobalt label can be measured over the liver after the technique of Glass, who showed that intrinsic factor is necessary for the detection of the vitamin

in pernicious anemia patients. The intrinsic factor has been shown to be a mucoprotein with unique biological activity secreted only by the gastric mucosa. However, not all the mucoprotein of gastric juice is intrinsic factor. Furthermore, there are numerous preparations of gastric juice or mucosa and other proteins exhibiting B_{12}-binding properties which possess little or no intrinsic factor activity.—C. R. SHUMAN

The microbiological methods provide another valuable tool in the study of the absorption and distribution of vitamin B_{12}. A rapid and simple procedure for this determination will be advantageous.

Rapid Estimation of the Serum Vitamin B_{12} Level by a Microbiological Method. R. H. Girdwood. *Brit. M. J.* 2: 954, 1954.

An estimate of serum vitamin B_{12} can be achieved in 16 hours by adding serum to a culture of *Lactobacillus leichmannii* and estimating the growth of the organism by turbidity measurements.

"The method is speedy, but suffers from the difficulties common to all methods of microbiological assay, and is probably more prone to technical troubles than is the *Euglena* (*gracilis*) assay."

Examples of the use of the method are given but there is only a brief general description of the method itself.—F. E. HYTTEN

The use of vitamin B_{12} as a supplement to improve the utilization of dietary protein has been the subject of several clinical studies. In experimental animals it has been demonstrated to have a positive effect upon growth rates and to increase the biological value of certain proteins.

Biological Value of Proteins as Influenced by Dietary Vitamin B_{12}. B. R. Baliga, S. Balakrishnan, and R. Rajagopalan. *Nature* 174: 35, 1954.

An increase in the utilization of soy bean proteins by rats occurred when vitamin B_{12} (50 μg. per kilogram of diet) or when Aureomycin (200 mg. per kilogram of diet) were added to the diet, even when the soy bean meal had been autoclaved. Casein was used as the source of protein in a second series of experiments, with the addition of either activated or inactivated proteolytic inhibitor prepared from soy beans and in equivalent amounts to that given by the soy bean diet. The active proteolytic inhibitor caused a drop in the utilization of casein, which could be reversed by the addition of vitamin B_{12} (50 μg. per kilogram of diet).

Vitamin B_{12} seemed to enhance the biological value of soy bean protein, but, in the quantities used, only succeeded in neutralizing the effect of proteolytic inhibitor on casein. No experiments were done to determine whether further vitamin B_{12} would enhance the normal biological value of casein.—F. E. HYTTEN

In pernicious anemia, the administration of vitamin B_{12} rapidly produces an improvement in appetite, lassitude, and weakness, even before the anemia is corrected. The reason for this is not clear; neither is there any explanation for the purported improvement in the patients with hepatitis treated with this agent.

The Effect of Vitamin B_{12} and Folic Acid in the Treatment of Viral Hepatitis. R. E. Campbell and F. W. Pruitt. *Am. J. Med. Sc.* 229: 8, 1955.

It has been suggested in previous studies that acute viral hepatitis can be improved by the administration of vitamin B_{12}. In the present study, two groups of 44 patients were treated with the usual program of dietary control and bed rest. The experimental group was given 30 mcg. of vitamin B_{12} intramuscularly every other day and 5 mg. of folic acid orally three times daily for the first 10 days of hospitalization. The two groups were entirely comparable with respect to age, duration of illness, and degree of icterus. It was observed that the group of patients receiving the vitamin B_{12} and folic acid had a more rapid return to normal appetite than the control group treated with diet and rest alone. The total serum bilirubin decreased to normal in 11 weeks in the experimental group compared with 18 weeks in the control group. The mean duration of illness was significantly shortened in the experimental group. The metabolic actions of vitamin B_{12} are reviewed with particular reference to its participation in the formation of thymine in nucleic acid synthesis through the transmethylation of uracil. In addition, it functions as a lipotropic and choline-sparing agent in experimental animals subjected to carbon tetrachloride or choline-deficient diets. These and other actions related to these vitamins may explain their benefit in hepatic repair and the improvement noted in hepatitis.—C. R. SHUMAN

NUTRITIONAL FACTORS IN ANEMIA

The common clinical anemias are those associated with iron deficiency due to chronic blood loss, or to chronic infection, together with an inadequate supply of this essential dietary element. In addition, the porphyrin and protein moieties of the hemoglobin molecule may not be produced when vitamin B_{12} or protein deficiencies are present, or when the erythropoietic tissue is affected by a wide variety of toxic factors and other disorders. The anemia of kwashiorkor is an interesting example of the influence of protein malnutrition upon bone marrow activity.

Anaemia in Kwashiorkor. E. B. Adams. *Brit. M. J.* 1: 537, 1954.

Blood changes were studied in 21 cases of kwashiorkor which were diagnosed only in the presence of pitting edema, dermatosis, and lowered plasma proteins. There was anemia in all cases and in 14 the

hemoglobin level was under 9 Gm. per 100 ml. During treatment, and parallel with a general clinical improvement, the anemia appeared to deteriorate, probably due to a rise in blood volume as the plasma protein level rose.

In 13 cases the anemia was normoblastic; in 6 cases the bone marrow showed giant metamyelocytes and some red cell precursors of intermediate type; and in 2 cases the bone marrow showed megaloblastic changes.

The details of hemoglobin, red cell count, mean cell volume, mean cell hemoglobin concentration, reticulocyte count, and white cell count are shown for each case. It is considered that the reticulocyte count is a more reliable index of recovery from anemia under treatment than the hemoglobin percentage.—F. E. HYTTEN

In scurvy, anemia is frequently encountered, not only because of hemorrhagic extravasations representing a source of blood loss, but also because of interference with hemoglobin formation.

Six Cases of Infantile Scurvy. G. Howells, P. E. S. Palmer, and W. H. St. John-Brooks. *Brit. M. J.* 2: 1143, 1954.

From an area of 250,000 inhabitants, six cases of scurvy in infants were seen during the years 1950–52. The cases are described in detail. They ranged from 7 to 12 months of age and had four features in common: limb tenderness varying from dislike of handling to acute pseudoparalysis; good general nourishment and development; radiological bone changes; and a rapid response to vitamin C. Anemia and swollen gums were each seen in three cases and petechial hemorrhages in only one case. All the babies had been artificially fed and the mothers of five admitted having given no supplementary orange juice. One child was said to have had regular orange juice.

Clinical diagnosis is usually straightforward, but radiology provides the quickest and easiest confirmation. It should be remembered, however, that the distribution of lesions is not constant, and all joints may need to be x-rayed.

An important lesson drawn from this series was that, although the diagnosis is theoretically easy, only one case was, in fact, diagnosed on first examination. The authors attribute this to the widespread belief that infantile scurvy no longer exists.—F. E. HYTTEN

The anemia of pregnancy is one in which the use of iron is of benefit, indicating the probability of a relative deficiency of the element due to increased requirements associated with this condition. In instances of nausea or gastric intolerance to iron therapy, intramuscular administration is indicated and appears to be effective.

Anaemia of Pregnancy Treated with Intramuscular Iron. J. M. Scott and A. D. T. Govan. *Brit. M. J.* 2: 1257, 1954.

Fifty pregnant women with iron-deficiency anemia were treated with intramuscular injections of a recently elaborated dextran-iron solution ("imferon"). In 14 of the women the hemoglobin was between 9 and 9.5 Gm. per cent, in 28 between 8 and 8.9, and in 8 less than 8 Gm. per cent. All anemias were of microcytic, hypochromic type, with normoblastic marrows. Some women were initially given injections equivalent to 100 mg. of iron daily, but finally all received 250 mg. twice weekly. There were no toxic reactions.

The hemoglobin rose slightly during the first 24–48 hours and then fell until about the fourth day, probably due to changes in the blood volume; subsequently it improved rapidly. The hematocrit reading and the mean cell volume increased from the beginning, and reticulocyte counts rose steadily to a maximum about the seventh day. Utilization of the iron was good: a rise of 0.3 Gm. hemoglobin was recorded for every 100 mg. of iron injected.

The serum iron rose to a maximum in about 36–48 hours. If the injection was given intravenously, there was a very high initial serum iron concentration which fell in 36–48 hours to levels comparable with those following intramuscular injection.—F. E. HYTTEN

Although oral iron therapy is the simplest and most practical method available for treatment of the iron-deficiency anemias, there is a continuing interest in the use of parenteral iron. The following papers attest to the efficacy of the dextran-iron preparation for intramuscular administration. The absence of toxic reactions is an important feature, as is the achievement of a hemoglobin rise of 0.3 Gm. per 100 mg. of injected iron.

A New Carbohydrate-Iron Haematinic for Intramuscular Use. D. F. Cappell, H. E. Hutchison, E. B. Hendry, and H. Conway. *Brit. M. J.* 2: 1255, 1954.

A small clinical trial with a newly elaborated dextran-iron hematinic ("imferon") is described. Fourteen patients with an iron-deficiency anemia were treated by intramuscular injection with the equivalent of 200 mg. of iron every second day; their hemoglobin values before treatment ranged from 4.3 Gm. to 9.8 Gm. per 100 ml. Six were known to be refractory to, and two had shown marked intolerance of, ferrous sulfate by mouth.

There were no toxic reactions, but staining of the skin at the site of injection occurred in a few cases; this could be avoided by injecting 1–2 ml. of air after the solution. Sustained serum iron levels of the order of 1500 μg. per 100 ml. were produced, with no appreciable iron loss in the urine. A satisfactory hematological response occurred in all cases, its speed being inversely proportional to the initial hemoglobin

level. The average weekly rise in hemoglobin during the first month ranged from 0.51 to 1.67 Gm. per 100 ml.

In one woman with mitral stenosis, who began treatment but died of a cerebral embolus from the left auricle, an opportunity was taken to study the method of iron absorption from the site of injection. The lymphatics and regional lymph nodes contained considerable quantities of iron, and it is suggested that absorption is by this route.—F. E. HYTTEN

Intramuscular Iron Therapy in Iron-Deficiency Anaemia. I. M. Baird and D. A. Podmore. *Lancet* 2: 942, 1954.

A dextran-iron preparation suitable for intramuscular injection has been studied in normal volunteers and in patients with iron-deficiency anemia. The solution is only one-third as toxic to mice as saccharated iron, is more stable, does not precipitate in plasma over a wide pH range, and is isotonic with tissue fluids; it contains 5 per cent iron.

The injection causes only moderate discomfort, but brown staining of the skin at the site of injection occurred unless a long needle was used and inserted in a Z-shaped track. There were no toxic reactions.

Serum iron levels were maximal from 1 to 2 days after injection but varied very widely: for example, two patients of similar weight and both with a hemoglobin of 8.9 Gm./100 ml. showed maximum serum iron levels of 1960 and 1000 μg. per 100 ml. after receiving 250 mg. of iron by injection. Normal serum iron levels were reached again 6 or 7 days after a single injection. Urinary excretion of iron was not increased at levels of serum iron up to 10.1 mg. (10,000 μg.) per 100 ml.

Thirty-eight cases of uncomplicated iron-deficiency anemia responded adequately to treatment with the intramuscular iron preparation, which was given in doses up to 300 mg. twice daily. It is calculated that 100 mg. of injected iron raises the hemoglobin by 0.34 Gm./100 ml. The maximum hemoglobin response was achieved in 4 to 9 weeks. The authors "feel that this preparation is effective and safe but should be restricted to iron-deficiency anaemia unresponsive to or intolerant of oral iron where adequate investigation has disclosed no other cause."—F. E. HYTTEN

The finding of high iron concentrations in the lymphatics following intramuscular administration of the hematinic (see above) is in contrast to the localization which occurs when iron is absorbed from the intestines; such iron appears primarily in the blood stream.

Lymphatics in Iron Absorption and Transport. N. B. Everett, W. E. Garrett, and B. S. Simmons. *Am. J. Physiol.* 178: 45, 1954.

These experiments demonstrate that the small amount of iron in lymph is derived from the blood stream after iron is given by gavage. Subcutaneous iron as ferric chloride is absorbed largely by blood vessels. Plasma-bound subcutaneous iron enters lymphatics almost completely. Intravenous iron appears rapidly in lymph which depends on binding of iron by plasma protein. White cells of blood and lymph do not play a role in iron absorption, no matter what route is used. It appears from the data obtained in this work that (in the rat) lymphatics do not play a role in absorption and transport of iron from intestine.—M. J. OPPENHEIMER

A Study of the Absorption and Utilization of an Iron Chelate in Iron-Deficient Patients. J. J. Will and R .W. Vilter. *J. Lab. & Clin. Med.* 44: 499, 1954.

The ferric-sodium salt of ethylenediaminetetraacetic acid (the latter is designated as a chelating agent, which refers to its tendency to form chemical bonds with certain cations) was compared to ferrous sulfate in relation to its metabolism and therapeutic efficacy in normals and in subjects with hypochromic anemia. Three approaches were: (1) Hemoglobin, erythrocytes, and reticulocytes were measured frequently in five patients with hypochromic anemia during the course of administration of subcorrective doses of ferrous sulfate over two weeks, followed by an equivalent dose of the chelated iron. (2) The rate of hemoglobin regeneration was estimated in three patients with hypochromic anemia given full doses of the chelated iron. (3) Ferrous sulfate and its chelated counterpart, each containing radioactive iron, were given to a few normal subjects and to one recovering from iron-deficiency anemia. Stool and urine were then studied for radioactivity. No striking differences in behavior of these two compounds (ferrous sulfate and chelated iron) were demonstrated, although experimental design may not have precluded neglect of significant differences. Their therapeutic efficacy was found to be similar.—R. TARAIL

PROTEIN SYNTHESIS AND DEGRADATION

The utilization of amino acids in the formation of body protein has been shown to be influenced by the caloric level of the diet. Increased nitrogen and amino acid utilization occurs with adequate calories supplied by carbohydrate and fat. However, increased protein allowances in subcaloric dietary regimens have been shown to reverse a negative nitrogen balance. The influence of dietary protein upon the plasma protein turnover has been studied in the following report.

Plasma Protein: V. The Effect of the Protein Content of the Diet on Turnover. H. L. Steinbock and H. Tarver. *J. Biol. Chem.* 209: 127, 1954.

Labeled plasma was prepared by administering methionine-S^{35} from S^{35}-labeled yeast to donor rats

which were sacrificed by withdrawing blood 8 hours later. The plasma was subjected to dialysis to remove any residual free labeled amino acid. The labeled plasma was injected into the external jugular vein of the experimental animals and blood samples were removed from the tail vein of each of the rats at intervals up to 7 days after the administration of the plasma. Three diets containing 0, 25, or 65 per cent protein were fed to the animals for a minimum of 3 weeks prior to the administration of the labeled plasma protein. The results of the experiments showed that the group of animals on the 25 per cent protein diet had a greater average pool size, a shorter half-life, and a greater replacement time than those on a diet free of protein. At these levels an increase in the protein content of the diet would result in an initial increase in the rate of plasma protein synthesis. A further change in the protein content of the diet from 25 to 65 per cent resulted in a decrease in the pool size, an additional decrease in the half-life, together with an increase in replacement rate. Here, an increase in the protein content of the diet resulted in an initial increase in the rate of degradation. Thus, whether a given change in the protein content of the diet will result in an initial stimulation of protein synthesis or of degradation will depend on the initial diet.

The authors conclude that the per cent protein in the diet greatly influences the half-life and the rate of replacement of the plasma protein in rats. On a protein-free diet replacement rate is small, whereas on a high protein diet it may be two or more times as high. One may obtain either an increase in rate of synthesis or in rate of degradation, depending on the nature of the original diet.—M. K. HORWITT

The stereoisometric configuration of amino acids may affect their intestinal absorption.

Selective Disappearance of Stereoisomers of Amino Acids From the Human Small Intestine. Y. Kuroda and N. S. Gimbel. *J. Appl. Physiol.* 7: 148, 1954.

The test object was a human patient with an isolated ileal loop of intestine. When racemic mixtures of amino acids were placed in the loop there were changes in the optical rotation of the solute after it had remained for one half hour. This seemed to indicate that the naturally occurring *l*-amino acids disappear from the loop more rapidly than their *d*-enantiomorphs.—M. J. OPPENHEIMER

The important problem of the most appropriate carbohydrate solution to be used for nutritional purposes involves the determination of its nitrogen-sparing action. This requires that the infused substance be rapidly utilized, thereby lowering its urinary excretion rate.

Protein-Sparing Action on Intravenously Administered Carbohydrate Solutions. A. A. Albanese, W. H. Arnold, D. R. Hays, A. Belmont, L. Orto, and R. DiFallo. *Metabolism* 3: 523, 1954.

It has been demonstrated that intravenously administered invert sugar exerts a superior protein-sparing action over that of dextrose in adults. In the present work, the authors record comparative data on the oncotic effects, nutritional availability, and nitrogen-sparing action of 10% solutions of invert sugar, and 5% and 10% solutions of dextrose and fructose. The nitrogen balances disclose that, at 50- to 100-Gm. levels of administration, fructose solutions induce greater nitrogen retention than invert sugar preparations with comparable quantities of fructose. However, the 10% solution of fructose resulted in poor nitrogen retention compared to that observed with the 5% concentration. Since one of the important objectives of parenteral fluid therapy is that of providing the greatest possible caloric input, the authors recommend the 10% invert sugar solution. The 5% fructose solution is also good but has a lower caloric value. The availability and low cost of invert sugar adds to its value as an infusion fluid.—C. R. SHUMAN

Immobilization and high environmental temperatures are two conditions leading to a loss of body protein manifested by a negative nitrogen balance. The basic metabolic disturbances producing this alteration are not clearly understood and are the subject of the following papers.

Isotopic Studies on the Mechanism of Negative Nitrogen Balance Produced by Immobilization. F. Schønheyder, N. S. C. Heilskov, and K. Olesen. *Scandinav. J. Clin. & Lab. Investigation* 6: 178, 1954.

This paper reports a study in which the mechanism of the negative nitrogen balance following immobilization was investigated by feeding N^{15}-labeled glycine. When *normal* subjects were placed in bed with both legs in a plaster cast, a negative N balance occurred after a lag period of five days and reached a maximum about the tenth day.

A much faster urinary excretion of N^{15} was found during the negative balance phase. By calculations based on nitrogen pools, it would appear that the mechanism for negative N balance during immobilization is a reduced synthesis of tissue proteins, while the rate of breakdown of these proteins remains unchanged.

Although these conclusions are based on certain theoretical assumptions, which the authors clearly demonstrate, this careful study has broad implications. Not only does it raise questions as to how this diminished synthesis of protein occurs, but it re-emphasizes the significance of adequate nutrition to those who must stay in bed.—S. O. WAIFE

Relationship of Hyperthermy to Nitrogen Excretion in Chickens. W. H. Edwards and W. O. Wilson. *Am. J. Physiol.* 179: 76, 1954.

After hyperthermy it was observed that urine contained a reduced percentage of non-protein nitrogen (uric acid nitrogen) and blood levels were also lower than controls. Urine nitrogen levels were increased. It is possible that hyperthermia acts upon some enzyme system which converts purines to uric acid in the fasted chicken.—M. J. Oppenheimer

PYRIDOXINE METABOLISM

Several reports have appeared in which a deficiency of pyridoxine has been found to produce certain symptoms such as convulsions and anemia in infants which responded rapidly to replacement therapy with the vitamin. The following paper will provide an excellent orientation in this subject for those interested in the clinical and experimental aspects of vitamin B_6.

Some Abnormalities of Vitamin B_6 Metabolism in Human Beings. R. W. Vilter, J. P. Biehl, J. F. Mueller, and B. I. Friedman. *Fed. Proc.* 13: 776, 1954.

Pyridoxine has been used in the treatment of nausea in pregnancy and in certain skin disorders, with negligible success. In infants, deficiency of this vitamin has produced both convulsions and anemia, both of which responded to replacement therapy. In adults, the use of a metabolic antagonist, desoxypyridoxine produced seborrheic dermatitis over the nasolabial folds, cheeks, chin, and scalp, sometimes extending to the shoulders or perineum. Glossitis and stomatitis were observed. Cheilosis, angular stomatitis, and conjunctivitis were noted. Several patients developed peripheral neuritis. Weight loss, weakness, anorexia, and depression occurred in many subjects. Lymphocytosis, anemia, and tendency to infections were prevalent findings.

The syndrome of pyridoxine deficiency in infancy, induced by diets limited to specially processed foods is described. A major report appeared in this journal (J. Clin. Nutrition 1: 200, 1953).

Some evidence has indicated an increased need for vitamin B_6 during pregnancy and lactation.

An important biochemical defect observed was that involving tryptophane metabolism. The administration of a 10-Gm. dose of tryptophane was associated with an abnormal rise in the urinary excretion of xanthurenic acid in pyridoxine-deficient subjects. The administration of isoniazid (INH) in the treatment of tuberculosis has been shown to adversely affect pyridoxine metabolism. Work has been presented to show that INH will form a conjugate with the vitamin resulting in its inactivation and excretion as pyridoxal isonicotinic hydrazone. If large doses of vitamin B_6 were given with INH therapy, no neuritic symptoms occurred; however, once the neuritis is present, it is necessary to discontinue INH therapy to produce a remission.—C. R. Shuman

Further details on the defective tryptophane metabolism in pyridoxine deficiency, adverted to in the preceding abstract, are emerging from the experiments of Italian workers.

Biochemical Lesions of Pyridoxine Deficiency and their Specificity. F. M. Chiancone, E. Ginouilhac, and L. T. Tenconi. *Acta vitaminol.* 8: 209, 1954.

Continuing their studies on experimental pyridoxine deficiency and its repercussions on the metabolism of tryptophane, the authors have investigated the specificity of the biochemical lesions produced by pyridoxine lack. The disturbed tryptophane metabolism noted in this condition is reflected in the altered ratio of its various end-products. Two metabolic routes are involved; from a common intermediate precursor, kynurenine, they lead, respectively, to the excretion of nicotinic bodies and of xanthurenic acid. In pyridoxine deficiency, urinary excretion of nicotinic acid and N′-methyl-nicotinamide decreases, while that of xanthurenic acid and kynurenine increases. An analogous pattern was reported by American authors in riboflavin deficiency as well, and the authors wished to precise the reaction of tryptophane metabolism to pyridoxine deficiency and observe the response to the administration of vitamin B_6 and B_2, respectively.

The urinary elimination of N′-methyl-nicotinamide, nicotinic acid, xanthurenic acid, kynurenine, and total diazotable substances was determined in rats on a pyridoxine-deficient diet after tryptophane only and after pyridoxine or riboflavin administration. Male albino rats, aged 30–40 days, were employed. Four animals (controls) were maintained for 36 days on a complete ration; 12 animals were maintained for the same period on a pyridoxine-free diet. On day 36, the rats were given a single oral dose of *l*-tryptophane (500 mg./Kg.). In addition, 4 of the deficient rats were given 250 mg. of pyridoxine hydrochloride, while 4 others received 250 mg. of riboflavin.

In the controls, only a rather small proportion of the tryptophane administered appeared in the urine in the form of the metabolites studied, and there was a definite relationship between kynurenine, on the one hand, and nicotinic bodies and xanthurenic acid, on the other—a relationship expressed as 1:0.4:0.75 (average).

In the pyridoxine-deficient rats, the percentage of the same metabolites with respect to the dose of tryptophane was much higher (4 to 5 times). The increase of kynurenine and xanthurenic acid accounted for this higher value, while the nicotinic bodies quota was reduced. Comparing the ratio with that obtained in the controls (see above), the authors found it to be 1:0.03:0.8.

When pyridoxine was given to the deprived rats, the values for this whole series of metabolites were

similar to those obtained in the controls, except that while kynurenine and xanthurenic acid approached the normal values, the nicotinic bodies remained at the level to which they had been reduced by pyridoxine deprivation.

As for the specificity of these findings, in pyridoxine-deprived rats receiving riboflavin instead of pyridoxine, the values obtained were similar to those of untreated pyridoxine-deprived controls (4 to 5 times higher than those of the non-deficient controls); their relationship was 1:0.03:1.2.

It is therefore apparent that the characteristic lesions observed after 36 days of a pyridoxine-deficient diet are not modified by riboflavin administration, whereas pyridoxine corrects the abnormality of the metabolic route tryptophane → kynurenine → xanthurenic acid. The effect of pyridoxine deficiency on this route is therefore both specific and reversible by pyridoxine. The deflection of the metabolic route terminating in the excretion of nicotinic bodies is, on the other hand, unaffected by pyridoxine, and may therefore be described as specific but non-reversible, at least in the conditions of the authors' experiments.—C.-J. HOWELL

Reports on the after-effects of pyridoxine deficiency in infants are beginning to appear. The following paper examines the status of the central nervous system in these infants.

A follow-up Study of Infants Fed a Vitamin-B_6-Deficient Diet. D. B. Coursin. *Am. J. Dis. Child.* 88: 803, 1954.

The author reports no residual central nervous system damage in a follow-up study ranging from 5 months to 2 years of an unstated number of infants who developed convulsive seizures due to vitamin B_6 deficiency. The electroencephalograms also were normal. They observed concentrations of 100 to 500 μg. per liter in a variety of undiluted milks and milk substitutes and caution that diluted formula products low in pyridoxine (vitamin B_6), in a subclinical manner, may be a contributing factor in certain cases of abnormal CNS development.

The abstracter agrees that minimal concentrations of vitamin B_6 should be present in milk products and milk substitutes. These studies indicate that it is unlikely for permanent brain damage to be associated with vitamin B_6 deficiency which has its onset as a result of inadequate ingestion of this vitamin postnatally. We shall await with interest similar follow-up studies of "pyridoxine dependency" resulting from prolonged administration of vitamin B_6 during pregnancy, which was reported by Hunt *et al.* and abstracted for this journal (Vol. 2, No. 6, p. 436).—J. N. ETTELDORF

While it has been shown by Bing and others that amino acids can be oxidized for energy formation by heart muscle, the following report suggests that there is no significant reduction of the respiration of cardiac tissue slices when the availability of amino acids for this purpose is lowered by pyridoxine deficiency.

Metabolism of Cardiac Muscle. VIII. Pyridoxine Deficiency. M. Brin, R. E. Olson, and F. J. Stare. *J. Biol. Chem.* 210: 435, 1954.

This paper deals with the effect of pyridoxine deficiency upon respiration, pyruvate and lactate utilization, and the activity of transaminase systems in duck heart ventricle. A pyridoxine deficiency in the duckling, sufficient to cause marked anemia, did not appreciably affect the respiration or the pyruvate or lactate utilization of duck ventricle slices *in vitro*, although a defect in transamination was demonstrable in homogenates of the same tissue. The pyridoxine deficiency depressed the activities of the glutamic-alanine and the glutamic-aspartic acid transaminases in the homogenates, 50 and 30 per cent, respectively. This transaminase activity of the deficient homogenates was stimulated by either the addition of pyridoxal phosphate *in vitro* or the administration of pyridoxine, pyridoxamine, or pyridoxal *in vivo*.

There was a relationship between the degree of anemia and the degree of depression of transaminase activity. The authors believe that the current study supports the general conclusion that although amino acids are essential for the anabolism of heart muscle protein, the contribution to the energy exchange of the intact heart by the transaminases is small in conditions of health, since the pyridoxine restriction can reduce transaminase activity in heart tissue without seriously affecting over-all respiration of slices *in vitro*.—M. K. HORWITT

Contribution to the Study of Transaminase Activity of Rat Liver Homogenates in Relation to Pyridoxine. L. T. Tenconi. *Acta vitaminol.* 8: 275, 1954.

In rats on a pyridoxine-deficient diet, the glutamic-alanine-transaminase activity of liver homogenate was reduced by 50 per cent. Pyridoxine administration returned this enzymatic activity to values corresponding to those of rats on a complete, balanced diet. Neither axerophthol (vitamin A) nor riboflavin modified the reduced transaminase activity values due to pyridoxine deficiency.—C.-J. HOWELL

In addition to functioning as a co-enzyme in the transaminase systems discussed above, pyridoxine is also involved in the enzymatic reactions associated with carbohydrate and fat metabolism.

Studies on the Effect of Vitamin B_6 Deprivation on Carbohydrate Metabolism in the Rat. J. R. Beaton and W. E. Goodwin. *Canad. J. Biochem. & Physiol.* 32: 684, 1954.

After five days of vitamin B_6 deprivation, rats have significantly lowered fasting levels of blood sugar and liver glycogen. Restriction of the vitamin for 21 days results in even more marked depression of these levels, accompanied by significantly lowered fasting blood levels of pyruvic and lactic acids. The livers of the vitamin B_6-deprived rats showed a diminished activity of acid dehydrogenase *in vitro*.

The findings support a hypothesis of an abnormal carbohydrate metabolism in vitamin B_6-deprived rats.—F. E. Hytten

Experimental findings on impaired endocrine function in pyridoxine deficiency have not as yet been clinically confirmed. Their implications are, however, of considerable interest.

Effect of Pyridoxine Deficiency on the Gonadotrophic Content of the Anterior Pituitary in the Rat. E. Wooten, M. M. Nelson, M. E. Simpson, and H. M Evans. *Endocrinology* 56: 59, 1955.

Pyridoxine (vitamin B_6) deficiency in rats results in a syndrome of impaired response to pituitary gonadotrophins and deficient formation of ovarian hormones. This was shown by Nelson, Lyons, and Evans. In the present report the pituitaries of vitamin B_6-deficient rats are assayed for gonadotrophic content. The pituitaries of normal and pyridoxine-deficient female rats were weighed, ground up, pooled, and injected as a suspension in isotonic saline. FSH and ICSH activity were assayed in hypophysectomized female rats. MH (mammotrophic) activity was assayed by the effect on crop sacs of squabs. The minimal effective dose (MED) was described by the number of pituitaries of the control and deficient rats required to produce a response in the test systems. The results can best be shown by this table.

Table 3. The Gonadotrophic Activity in the Anterior Pituitaries of Normal and of B_6 Deficient Female Rats

Type of animal	FSH activity MED	ICSH activity MED	MH activity MED
	no. pits	*no. pits*	*no. pits*
Normal	5	1	1/32
B_6-deficient	3/4	3/4	1/32

The pyridoxine-deficient animals showed 7 times as much FSH activity per gland and 10 times the FSH activity per milligram of tissue. The ICSH activity was only slightly higher in the deficiency animals and the MH was unchanged. These observations suggest either a decreased end-organ response to gonadotrophins and/or a decreased release of gonadotrophins from the pituitary. It is of interest that impaired end-organ response to hormones has been demonstrated in other vitamin deficiencies, that is, folic acid deficiency.—L. Recant

One of the research tools employed in studying pyridoxine metabolism has been de(s)oxypyridoxine, a metabolic antagonist for the vitamin. Certain differences observed between the vitamin B_6-deficient animals and those receiving the antivitamin are recounted below. (These findings are reminiscent of differences observed in the types of response to thiamine deficiency produced by different vitamin B_1 antagonists.)

The Effect of B_6 and Deoxypyridoxine on Urea Formation in Rats. J. R. Beaton, M. E. Goodwin, G. Ozawa, and E. W. McHenry. *Arch. Biochem. & Biophysics.* 51: 94, 1954.

The experiments described are an extension of earlier observations on elevated urea levels during vitamin B_6 deficiency induced by using the anti-vitamin deoxypyridoxine in an effort to determine whether this compound, which markedly accentuates the external deficiency signs of pyridoxine deficiency, has a similar effect *in vitro*. Forty rats were divided into four groups. The first group received the basal diet *ad libitum* supplemented with deoxypyridoxine. The second group received the basal diet supplemented with deoxypyridoxine and pyridoxine. The third group received the basal diet unsupplemented, and the fourth group received the basal diet supplemented with pyridoxine. The food intake of the last three groups was restricted to that of the first. The animals were maintained on experimental feeding for a period of 28 days, the average daily food intake of all groups being 6 Gm./rat. At the end of this period of experimental feeding, every animal in the group provided with deoxypyridoxine but no pyridoxine exhibited the acrodynia of vitamin B_6 deficiency. None of the rats in the remaining groups exhibited any evidence of skin lesions.

Examination of urea formation by liver slices of these animals confirmed the previous observations that the vitamin B_6-deprived rats had significant increases in urea formation. In the deoxypyridoxine animals, this abnormality was not accentuated.

No significant elevation in liver arginase activity was observed in the vitamin B_6-deprived rats which had been given dietary deoxypyridoxine. However, deoxypyridoxine appeared to restore the mean liver arginase activity of deprived animals to that of the controls, even though the external deficiency signs were accentuated. It is suggested that deoxypyridoxine is not a true anti-vitamin in accentuating biochemical abnormalities, even though the external modifications of the deficiency are obtained.—M. K. Horwitt

ITEMS OF GENERAL INTEREST

Therapeutic Nutrition with Tube Feeding. M. D. Pareira, E. J. Conrad, W. Hicks, and R. Elman. *J. A. M. A.* 156: 810, 1954.

Some type of tube feeding has been used since the seventeenth century. There has been a persistent quest for an improved formula which would sustain nutrition and be practicable and acceptable. This study presents the observations on 240 patients, most of whom were hospitalized and fed by tube only for from one to 16 weeks.

The indications were anorexia due to active diseases (such as tuberculosis, cerebrovascular accidents, cancer, trauma), anorexia nervosa, post-convalescent malnutrition, mechanical impediments to eating or swallowing, semi- or unconsciousness, and preoperative preparation.

A polyvinyl tube with an open tip was introduced into the stomach. It has been left in place for as long as four months without clogging. The mixture was introduced either intermittently by a 50-ml. syringe, or by slow drip.

The mixture was composed of powdered whole milk, nonfat milk solids, calcium caseinate, dextrin, and maltose, vitamins and minerals, suspended in water.

Nine hundred grams of the mixture furnished 3500 cal., 210 Gm. protein, 600 Gm. carbohydrate, and 30 Gm. fat, plus the recommended dose of vitamins. The details of the administration are given in the paper.

The preparation was well tolerated. Diarrhea developed in only seven per cent, and was severe in only two per cent of the total group. All patients studied had a positive N balance and most gained weight. Anorexia was corrected in nearly all patients after several weeks of tube feeding. No evidence of aspiration was seen.

This is a valuable paper and represents a long, careful study. It would appear that prolonged tube feeding is practicable and acceptable.—S. O. Waife

Allergy to Foods Containing Sulfur-amino-acids, Especially Methionine: Case Report and Clinical Study. L. Tuft, L. N. Ettelson, and H. Schwartz. *Am. J. Med. Sc.* 229: 26, 1955.

Allergic manifestations, including rhinitis, headache, cough, and sneezing, were found to be attributable to foods high in sulfur content in a 27-year-old man. Clinical testing clearly established the nature of the sensitivities; however, direct skin testing or passive transfer experiments proved to be negative in this case. The ingestion of methionine, a sulfur-containing amino acid present in large amounts in egg and other foods, resulted in the occurrence of typical allergic symptoms. This phase of the investigation was conducted as a blind study in which three types of capsules were used, one containing methionine, one cysteine, and one an inert substance. The methionine-containing capsule was found to be the one responsible for the recurrence of symptoms. To eliminate the intestinal organisms which may convert methionine to a mercaptan product, a sulfa-aureomycin preparation was administered. During this period, methionine did not produce allergic symptoms, while the inhalation of allergens arising from frying eggs resulted in allergic manifestations. This may represent an instance of food allergy resulting from sensitivity to the split products of protein digestion with positive clinical histories and negative skin reactions.—C. R. Shuman

The Malnourished Community. Care of Mothers and Children as a First Step Towards Improved Feeding. B. S. Platt. *Lancet* 2: 929, 1954.

In general terms it is suggested that, as a first step toward promoting social and economic development among unsophisticated peoples, the welfare of women and children should be fostered.

The women produce and process the food crops, and schemes to improve food production acceptable to them must be introduced. A small power unit for lighting, water pumping, and milling, run on local fuel, is a basic necessity. It is further suggested that the status of women should be improved by banding them into social units on the lines of "Women's Institutes," and that family limitation should be introduced as part of the improvement in the woman's economic status.

Infant welfare is also important, and much of it can be implemented through the mothers. "An immediate consequence of better feeding of the mother would be that she would produce ample milk daily and during a prolonged lactation." It is stressed that, when making plans in infant welfare, local tradition, particularly in feeding customs, should be respected.

(This is a very general article of little practical value; much of it is based on opinion rather than fact.)—F. E. Hytten

Food Taboos in East Africa. H. Trant. *Lancet* 2: 703, 1954.

"The first aim of a nutrition survey should be to find out why the people, or some of the people, are undernourished; and one cause of malnutrition which investigators who have not lived in the tropics are apt to overlook, is food taboos."

Food taboos appear to be widespread in East Africa. Some probably began as public health measures; others are related to totem observance, i.e., a tribe with a wild pig as a totem does not eat that animal; others concern animals used for sacrificial purposes; but the origins of many are lost in history.

Women, particularly when pregnant, are subject to considerably more taboos than men and children. The pregnant East African woman is forbidden, among many other foods, eggs, milk, and most meats.

The customs vary from tribe to tribe, and the author gives a number of detailed examples. She considers that although the rural Africans are still under the sway of food taboos, teaching by ante-natal clinics and baby clinics could do much to break them down.—F. E. Hytten

The Nutrition of Schoolgirls in Northern Nigeria. D. C. Wilson. *Brit. J. Nutrition* 8: 83, 1954.

Information was collected about the customary diets of eight main racial groups in Northern Nigeria: (a) peoples belonging to the North-Plateau pagans including Beroms, Shuwa Arabs, Nupe, Hausa, Settled Fulani and Kanuri; (b) two racial groups, Ibo and Yoruba, immigrants from the south, now living in the northern territory.

The observations were made late in the dry season, after a period of food shortage. A higher incidence of poor nutriture and thinness was found in children attending day schools than in school boarders whose food intake was more assured. Among day scholars, nutrition was poorer in the urban groups of southern immigrants than among the more favorably placed Nupe, who had an agricultural background. The degree of umbilical hernia was closely associated with poor nutriture and slackness of the abdominal muscles, though the origin of the condition must be sought in the indigenous methods of treatment of the umbilical cord at parturition.

Seasonal deficiency of ascorbic acid was suggested, especially among the Hausa and pagan tribes, by the red, swollen, and bleeding gums, which could not primarily be attributed to defective hygiene. The severity of lesions of the skin, of the lips, and of the eyes must be ascribed to the dusty dry climate, although in some cases inflammation may have been aggravated by a low intake of vitamin A. These conditions were most evident among the very primitive Beroms who lived in very dirty round huts, full of smoke. The degree of dental caries varied inversely with the degree of dental fluorosis.—B. Sure

Energy Expenditure in Bicycle Riding. D. B. Dill, J. C. Seed, and F. N. Marzulli. *J. Appl. Physiol.* 7: 320, 1954.

In this study, the energy requirement for bicycle riding was evaluated in terms of cross-sectional diameter of the tires. Studies were carried out at inflations which were recommended. Under this set of circumstances, the net oxygen consumption is 0.19 L./min. greater on 26 x $2^1/_8$-inch tires than on 26 x $1^1/_4$-inch tires. Larger tires, which are provided on American bicycles, require a greater energy expenditure than do those on European bicycles, where the tires are smaller in most cases.—M. J. Oppenheimer

Vitamin A Therapy in Oligospermia. H. W. Horne, Jr., and Charlotte L. Maddock. *Fert. & Ster.* 3: 245, 1952.

High oral doses of an aqueous dispersion of vitamin A were given to 12 patients with spermatogenic deficiency. All patients received 100,000 units per day for a twelve-week period; and from the eighth week on, 3 were given daily a supplemental dose of vitamin E, 5 an additional 100,000 units of vitamin A daily, the remaining 4 receiving no supplements. There was a rise of 30 per cent in the circulating vitamin A level, and the groups receiving supplements of vitamin E or additional vitamin A showed some increase over their previous eight-weeks' blood vitamin A level; but no change was noted in carotenoid, cholesterol, or lecithin blood levels, nor in basal metabolic rates. The cholesterol/lecithin ratio—abnormally high before therapy—dropped toward normal during the experiment. Except for one case, in which sperm output increased inexplicably after therapy, spermatogenesis was not affected.

It would seem, therefore, that vitamin A—at least in the doses used—is as conspicuously unsuccessful in the treatment of oligospermia as most of the agents heretofore employed in an attempt to correct it.—C.-J. Howell

A Study of the Nature of Glycogen Regeneration in the Intact Animal. M. R. Stetten and DeWitt Stetten, Jr. *J. Biol. Chem.* 207: 331, 1954.

Previous studies on the turnover of tissue glycogen in intact animals have depended upon the assumption that glycogen was metabolically homogeneous and that isotopes appearing in glycogen were uniformly distributed among glucose residues in various portions of the molecule. This study investigates the possibility that the peripherally situated glucose residues can be replaced without affecting the turnover in a stable central core. To test the latter thesis, glucose-C^{14} was administered to fasting rats and the glycogen from the liver and the carcass was analyzed at various periods from 3 to 48 hours after the radioactive glucose was injected. Barley β-amylase was used for enzymatic splitting of the liver glycogen samples into maltose and limit dextrin.

In all experiments lasting 6 hours or less, the specific activity of the glucose residues into peripheral chains was higher than that in the limit dextrin (glycogen, β-amylase). By the twelfth hour this relationship was reversed, higher specific activities being found in the limit dextrin than in the peripheral glucose residues. A contrasting situation was encountered in the glycogen of the carcass, where, even after 48 hours, the major fraction of the labeled glucose was found in the peripheral tier.

These findings show that glycogen, although yielding only glucose upon total hydrolysis, is metabolically inhomogeneous. Glucose residues situated peripherally in the glycogen molecule are renewed faster than those more centrally located. This contrast is more striking in muscle than in liver.

While this paper on glycogen labeling and that reported by Steinberg and Anfinsen (J. Biol. Chem. 199: 25, 1952) on non-uniform protein labeling are excellent examples of the specificity of isotope techniques, they demonstrate the possible danger of oversimplified interpretations of metabolic data from experiments with isotopes.—M. K. Horwitt

Milk, p-Aminobenzoate, and Malaria of Rats and Monkeys. F. Hawking. *Brit. M. J.* 1: 425, 1954.

Rats fed on a milk diet, or other diet deficient in *p*-amino-benzoate, are insusceptible or only slightly susceptible to infection by *Plasmodium berghei*, but become susceptible when 20 mg. of *p*-amino-benzoate per kilogram body weight per day is added. The deficiency could also be corrected by the addition of folic acid, but not by vitamin B_{12}, methionine, or *p*-hydroxybenzoate.

Monkeys maintained on a milk diet were insusceptible to infection by *P. knowlesi* or *P. cynomolgi*, but became fully susceptible when *p*-aminobenzoate was added in amounts of 0.5 mg. per kilogram body weight daily.

p-Aminobenzoate appears to be a growth factor for many species of malaria parasite.

Baby rats and baby monkeys, while exclusively breast-fed by their mothers are insusceptible to infection with *P. berghei* or *P. cynomolgi*, respectively, provided that their mothers are fed a *p*-aminobenzoate-deficient diet.

It is suggested that the relative immunity to malaria shown by infants under 6 months in many parts of the tropics may be due to a deficiency of *p*-aminobenzoate in their milk diet.

There is considerable experimental detail given, and any worker in this field should read the paper in its entirety.—F. E. HYTTEN

Body Water at Sea Level and at Altitude. W. E. Siri, C. Reynafarje, N. I. Berlin, and J. H. Lawrence. *J. Appl. Physiol.* 7: 333, 1954.

The mean values for body water are normal at sea level and at 16,000 feet. The subjects were normal young adults. The means values fell into the normal area for their ages and occupations.—M. J. OPPENHEIMER